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Paleolimnological assessment of long-term water-quality changes in south-central Ontario lakes affected by cottage development and acidification

Roland I. Hall and John P. Smol

Abstract: Sediment diatom assemblages were used to evaluate water-quality changes since preindustrial times in south-central Ontario lakes receiving acidic deposition and moderate shoreline (mainly cottage) development. Canonical correspondence analysis identified significant relationships between surface sediment diatoms and environmental factors. Relationships were sufficiently strong to develop weighted-averaging regression–calibration models for inferring lake water pH and total phosphorus concentration ([TP]) from diatoms. These models were accurate to within ±0.21 pH units and ±4.2 μg TP·L^{-1}. Postindustrial pH and [TP] changes were inferred from surface and pre-1850 sediment diatom assemblages. Results suggest that presently acid lakes (pH < 6) have acidified, and the pH of alkaline lakes (pH > 7) has increased, as observed in other regions receiving elevated acidic deposition. Diatom-inferred [TP] suggests that cultural eutrophication has not been widespread. In almost all lakes, present-day [TP] is not higher than before European settlement. In many mesotrophic lakes, preindustrial [TP] was higher than at present. Several factors could account for declining lake water [TP], including (*i*) lake and (or) watershed acidification processes that reduce P loading, or increase P loss rates; and (*ii*) reductions in nutrient loading from watersheds as a result of reforestation.

Résumé : Nous nous sommes servis des assemblages de diatomées dans les sédiments pour évaluer les changements de la qualité de l'eau, depuis la période préindustrielle, dans des lacs du centre-sud de l'Ontario qui ont connu des dépôts acides et un développement du littoral modéré (principalement construction de chalets). L'analyse de correspondance canonique a relevé des relations significatives entre la présence des diatomées dans les sédiments superficiels et les facteurs environnementaux. Les relations étaient suffisamment fortes pour que nous élaborions des modèles de régression–calibration à moyenne pondérée permettant de calculer le pH de l'eau du lac et la concentration de phosphore total ([PT]) à partir des diatomées. Ces modèles étaient exacts jusqu'à ±0,21 unité de pH et ±4,2 μg PT·L^{-1}. Nous avons reconstitué les changements du pH et du [PT] depuis la période postindustrielle à partir des assemblages de diatomées dans les sédiments de la surface et dans les sédiments antérieurs à 1850. Les résultats permettent de penser que les lacs présentement acides (pH < 6) se sont acidifiés, et que le pH des lacs alcalins (pH > 7) a augmenté, comme on l'a observé dans d'autres régions qui ont reçu des dépôts acides en grandes quantités. Le calcul de [PT] à partir des diatomées permet de penser que l'eutrophisation causée par les cultures n'était pas répandue. Dans presque tous les lacs, le [PT] n'est à l'heure actuelle pas plus élevé qu'avant la colonisation européenne. Dans de nombreux lacs mésotrophes, le [PT] préindustriel était plus élevé qu'il ne l'est maintenant. Plusieurs facteurs peuvent expliquer cette baisse du [PT] de l'eau des lacs, notamment (*i*) les processus d'acidification du lac et (ou) du bassin versant qui réduisent la charge en phosphore, ou accroissent les déperditions de phosphore et (*ii*) les réductions de la charge en nutriments dues au reboisement des bassins versants.
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Introduction

The majority of ecological studies evaluating ecosystem responses to anthropogenic stress have focused on large-scale perturbations, or so-called sledgehammer blows (Schindler 1987). Examples include large-scale increases in nutrient and acid loading at the Experimental Lakes Area (Schindler et al. 1973, 1980, 1985); acid and metal deposition at Sudbury, Ontario (Freedman and Hutchinson 1980); and clear-cutting and herbicide treatment of whole ecosystems (Likens et al. 1970; Bormann et al. 1974). Low-level perturbations have received relatively little or no attention.

Most Canadian lakes occur within the Precambrian Shield, and most are relatively remote from major urban and industrial centres. These remote lakes and their watersheds receive at least low to modest levels of multiple anthropogenic stressors, including elevated acid and metal deposition from industrial emissions (Neary et al. 1990) and elevated nutrient loading, mainly from cottages, associated septic wastes, and other shoreline activities (Dillon and Rigler 1975; Hutchinson et al. 1991; Dillon et al. 1993). However, it is not known whether low levels of multiple stressors have resulted in water-quality and biological changes in the vast majority of Canadian lakes (Schindler 1987).

The optimal method for assessing the severity of ecosystem damage is with reference to preimpact conditions (Smol 1992). Direct historical measurements are the preferred sources of such information, but they are rarely available (Likens 1989), especially for lakes in remote regions. As a result, we must rely on indirect sources of information to infer long-term trends in water quality.

Paleolimnological techniques, using diatom assemblages as biomonitors of aquatic change, provide an effective approach to supply missing historical data (Battarbee et al. 1990; Dixit et al. 1992*a*; Charles et al. 1994; Dixit and Smol 1994). Sedimentary diatom assemblages have been used successfully to evaluate water quality trends resulting from (*i*) lake acidification (Charles et al. 1989; Birks et al. 1990*a*; Dixit et al. 1992*b*, 1992*c*; Cumming et al. 1992), recovery (Dixit et al. 1992*b*, 1992*c*, 1992*d*), and associated changes in metals (Birks et al. 1990*a*; Dixit et al. 1992*c*, 1992*d*; Kingston et al. 1992) and concentrations of dissolved organic carbon ([DOC]) (Birks et al. 1990*a*); (*ii*) lake eutrophication (Anderson et al. 1993; Christie and Smol 1993; Hall and Smol 1993; Anderson and Rippey 1994; Bennion 1994; Zeeb et al. 1994; Reavie et al. 1995); and (*iii*) salinity associated with climatic change (Fritz 1990).

In Precambrian Shield regions, paleolimnological studies have investigated water quality changes at Sudbury, Ontario (Dixit et al. 1992*b*, 1992*c*, 1992*d*), and the Adirondacks, New York (Charles et al. 1990; Cumming et al. 1992). These studies assessed only trends in lake water acidity and metals resulting from acidic precipitation. To date, there have been no large-scale paleolimnological analyses of water-quality changes caused by other human activities, such as shoreline development, which may alter nutrient supplies to large numbers of Precambrian Shield lakes. As a result, their long-term impacts on lake water quality remain largely unknown.

In this study, we evaluate water-quality and biological changes that have occurred since preindustrial times in relatively remote, not heavily affected Precambrian Shield lakes in south-central Ontario, using sediment diatom assemblages. To do this, we first explore the relationships between surface sediment diatom assemblages and environmental gradients in a suite of Muskoka-Haliburton lakes. Second, we develop models for inferring quantitative water-quality trends from sediment diatom assemblages. Third, we use these diatom inference models to estimate changes in total phosphorus concentrations ([TP]) and lake water pH that have occurred between the present day and preindustrial (i.e., pre-1850) times. This study provides an overall assessment on how south-central Ontario lakes have responded to acidic deposition and shoreline development, including mainly cottages and past logging.

Materials and methods

Approach

An effective approach to studying diatom communities with respect to lake water quality is to analyze the surface-sediment diatoms from a set of lakes with complete and reliable limnological data, the so-called calibration set approach (Charles and Smol 1994). The surface sediments (e.g., top 1-cm interval) contain an integration of diatoms from a variety of lake habitats that have accumulated during the past few years. These surface sediments contain a wealth of information that can then be used to infer past water-quality conditions, on the basis of the percent abundances of sedimentary diatom taxa (Charles et al. 1990; Birks et al. 1990*a*, 1990*b*; Dixit et al. 1992*a*).

Detailed assessments of anthropogenic impacts on lake water quality require continuous temporal records of diatom assemblages. However, it is logistically prohibitive to analyze complete sediment cores for a large population of lakes. For large-scale studies aiming to provide regional estimates of limnological change, the most effective approach is to analyze microfossils in present-day and preindustrial sediment core samples (Charles and Smol 1990; Cumming et al. 1992). Differences between preindustrial and present-day inferences estimate water-chemistry changes caused by anthropogenic activity and also provide information on background or reference conditions. This approach has been successfully used to provide regional estimates of lake water chemistry changes in the Adirondacks, New York (e.g., pH, acid-neutralizing capacity (ANC), monomeric [Al] and [DOC]; Sullivan 1990; Sullivan et al. 1990; Cumming et al. 1992), Sudbury, Ontario (pH, total [Al], [Ni], [Ca]; Dixit et al. 1992*b*), and the northeastern U.S.A. (pH, [TP], [Cl], Secchi depth; Dixit and Smol 1994).

Study area and lake selection

The 54 study lakes are all situated within the Muskoka-Haliburton region of south-central Ontario, Canada (Table 1), a largely undeveloped area underlain mainly by Precambrian (Grenville Province) bedrock (Chapman and Putnam 1984). Local topography is rugged. Rounded knobs and ridges, usually between 20 and 100 m high, are separated by lakes and swampy lowlands. Tills and soils are generally shallow, but locally thicker clay, sand, and gravel deposits occur. The nutrient-poor, acidic, podzolic soils have low ANC (Chapman and Putnam 1984). The regional climate is cool, with an average of 189 frost-free days per year. Mean annual January and

Table 1. List of the 54 study lakes with selected geographical and physical data.

No.	Name	Latitude (N)	Longitude (W)	Surface area (ha)	Watershed area (km^2)	Maximum depth (m)
1	Twelve Mile	45°01′	78°43′	350	89 658	25.0
2	Three Mile	45°10′	79°27′	625	12 735	4.5
3	Bass	45°07′	79°42′	88	487	7.0
4	Basshaunt	45°07′	78°28′	47	697	24.0
5	Bigwind	45°03′	78°04′	111	416	32.0
6	Blue Chalk	45°11′	78°56′	52	158	23.0
7	Boshkung	45°04′	78°44′	716	87 908	71.0
8	Bruce	45°11′	79°40′	97	311	7.5
9	Buck	45°22′	79°00′	40	242	30.0
10	Butterfly	45°05′	79°38′	61	229	6.0
11	Canning	44°57′	78°38′	244	28 653	22.3
12	Chub	45°13′	78°59′	34	306	27.0
13	Clear	45°11′	78°43′	88	253	33.0
14	Crosson	45°05′	78°02′	57	578	25.0
15	Dickie	45°09′	79°05′	94	500	12.0
16	Eagle	45°08′	78°29′	235	21 036	24.4
17	Frazer Island (L. Joseph)	45°15′	78°46′	78	42 088	29.0
18	Gravenhurst Bay (L. Muskoka)	44°56′	79°24′	179	1 149	15.2
19	Green	45°07′	78°37′	131	49 170	12.2
20	Gullfeather	45°06′	79°01′	66	814	13.0
21	Haliburton	45°13′	78°25′	1014	1 340	54.9
22	Hall's	45°06′	78°44′	543	27 190	80.5
23	Hamer Bay (L. Joseph)	45°13′	79°46′	119	42 089	46.9
24	Hammel Bay (Three Mile L.)	45°10′	79°27′	153	1 349	13.0
25	Harp	45°23′	78°08′	71	542	37.5
26	Heney	45°08′	79°06′	21	752	5.8
27	Kashagawigamog South Basin	45°01′	78°34′	518	23 699	35.0
28	Kashagawigamog North Basin	45°01′	78°34′	315	20 986	22.9
29	Kelly	45°15′	78°37′	99	3 307	31.0
30	Kennisis	45°13′	78°39′	1417	13 412	68.0
31	Kushog North Basin	45°08′	78°50′	184	3 868	38.1
32	Kushog South Basin	45°06′	78°48′	444	7 308	26.0
33	Little Clear	45°24′	79°01′	11	149	25.0
34	Little Redstone	45°13′	78°34′	226	13 837	62.2
35	Little Kennisis	45°15′	78°36′	231	6 734	43.9
36	Leech	45°03′	79°06′	82	276	13.7
37	Leonard	45°04′	79°27′	195	440	15.2
38	Maple	45°06′	78°40′	336	5 100	36.6
39	McKay	45°04′	78°10′	122	568	19.5
40	Moose	45°09′	78°28′	290	18 219	43.9
41	Muskoka Bay (L. Muskoka)	44°57′	79°24′	241	1 149	13.5
42	Oblong	45°11′	78°26′	91	15 118	27.5
43	Plastic	45°11′	78°50′	32	128	16.3
44	Red Chalk East Basin	45°11′	78°56′	13	589	19.0
45	Red Chalk Main Basin	45°11′	78°56′	44	589	38.0
46	Red Pine	45°12′	78°42′	365	17 729	38.7
47	Redstone	45°12′	78°32′	1130	18 983	82.4
48	Roothog Island (Lake of Bays)	45°15′	79°00′	1675	39 661	79.2
49	Seagull Rock (Lake of Bays)	45°15′	79°00′	903	39 661	57.2
50	Solitaire	45°24′	79°01′	124	283	31.0
51	Soyer's	45°01′	78°37′	333	4 665	50.0
52	St. Nora	45°09′	78°50′	264	1 798	39.0
53	Walker	45°24′	79°05′	68	206	17.0
54	Young	45°12′	79°33′	106	211	21.1

July temperatures are 11.0 and 17.7°C , respectively. Precipitation amounts to 90–110 cm·yr^{-1}, 24–30 cm of which falls as snow.

The study area lies within the Great Lakes – St. Lawrence Forest Region (Rowe 1977), characterized by a mixed deciduous and coniferous forest dominated by sugar maple (*Acer saccharum*), beech (*Fagus grandifolia*), yellow birch (*Betula lutea*), red maple (*Acer rubrum*), eastern hemlock (*Tsuga canadensis*), and white and red pine (*Pinus strobus* and *Pinus resinosa*). The landscape is predominantly forested and nonagricultural, owing to the rough topography, thin soils, and cool climate. Several rural municipalities exist in the region, but seasonal recreation is the dominant human activity. The shores of most lakes are dotted with cottages, and most are serviced with septic sewage systems. Clear-cut logging was a major industry of the past, with the last major forest clearing 60–100 years ago. Agricultural activity is almost nonexistent, except in the vicinity of Three Mile Lake.

Lakes were selected from data bases maintained at the Ontario Ministry of Environment & Energy (OMOEE), Dorset Research Centre. Lakes were specifically chosen to sample the broadest trophic status gradient possible, and they ranged from very oligotrophic (spring overturn [TP] = 2.7 μg·L^{-1}) to mesotrophic (24.3 μg·L^{-1}) (Table 2). Few, if any, eutrophic lakes exist in this region. To minimize the influence of pH and pH-related variables on the distribution of diatom taxa, we did not include lakes with pH < 5.5 or with [DOC] > 6.5 mg·L^{-1}. Otherwise, the lake set included broad gradients of physical and water chemistry characteristics typical of this region (Tables 1, 2).

Field and laboratory methods

Sediment cores were collected during autumn 1992 from the deepwater regions of the 54 lakes using a Glew (1989) gravity corer fitted with a 6.6 cm internal diameter Lucite tube. To minimize stratigraphic disturbance, sediment cores were sectioned at the lake shore immediately after collection using a vertical extruder (Glew 1988). Present-day (0–1 cm interval) and preindustrial (1 cm thick interval from >20 cm core depth, and usually >25 cm) sediment samples were removed from the cores. We are confident our core bottom samples represent preindustrial (i.e., pre-1850) conditions in all lakes, because ^{210}Pb sediment dating from large numbers of south-central Ontario lakes indicates that a sediment depth of 15 to 20 cm almost always represents the pre-1850 period (Evans and Dillon 1982; Dillon et al. 1986; Evans et al. 1986; Uutala et al. 1994). ^{210}Pb dating from other Precambrian Shield regions indicates that preindustrial sediment intervals are usually located within the top 20 cm (e.g., Adirondacks: Charles et al. 1990; Cumming et al. 1992; Norton et al. 1992; Sudbury: Dixit et al. 1992*b*).

Lake water sampling (Locke and Scott 1986) and chemical analyses (Ontario Ministry of Environment 1983; Janhurst 1994) were performed by OMOEE personnel according to standard methods. Water samples were unfiltered, and therefore chemistry measurements are total concentrations including both dissolved and particulate fractions. Total phosphorus concentrations were averaged from samples taken during the spring overturn period. All other chemical variables were determined from annual averages of volume-weighted epilimnetic or metalimnetic composites, sampled biweekly, monthly, or bimonthly during the ice-free seasons from 1990 to 1992 (Table 2). Thus, water chemistry data correspond to multiple measurements collected during the period when diatoms in the top 1-cm sediment sample were most likely deposited. In a few cases, lake sampling was less frequent; data for Three Mile Lake, Hammel Bay, Bass Lake, Bruce Lake, and Butterfly Lake were averaged from volume-weighted epilimnetic samples collected twice, during November 1992 and November 1994.

Diatom slides were prepared from the top and bottom samples of each core using standard techniques described by Hall and Smol (1992). For each sample, a minimum of 400 diatom valves was identified and enumerated along transects using a Nikon Optiphot microscope fitted with differential interference contrast optics (×1000 magnification, numerical aperture = 1.25). A Hitachi S-2500 scanning electron microscope was used to study taxonomically difficult species. Diatom taxonomy generally followed Hustedt (1930), Patrick and Reimer (1966, 1975), Koppen (1975), Germain (1981), Camburn et al. (1984–1986), Krammer and Lange-Bertalot (1986–1991), and Cumming et al. (1995).

Sediment samples are stored at the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Department of Biology, Queen's University, Kingston, Ont. A set of microscope slides and diatom slurries are archived in the Algal Collection, Canadian Museum of Nature, Ottawa, Ont. (accession Nos. 38 630 – 38 739).

Data analysis

Diatom taxa were included in ordinations and the development of calibration models if they were present in a minimum of five lakes and achieved ≥1% abundance in at least one lake. Of the 416 taxa identified from the 54 surface sediment samples, 92 met these requirements. These taxa contributed 73–99% (average = 90.7%) of the total frustules counted in the surface sediment samples and 59–99% (average = 82.2%) of those in the bottom samples.

Ordinations were performed using CANOCO version 3.12 (Ter Braak 1988*a*, 1990). In all ordinations, rare taxa (i.e., taxa with percent abundances less than one fifth of the most common taxon) were downweighted in proportion to their frequency (Ter Braak 1988*a*). Principal patterns in the distributions of surficial-sediment diatom taxa were explored using detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA). DCA, with detrending by segments and nonlinear rescaling, was used to determine the maximum amount of variation in the species data (Hill and Gauch 1980). CCA, a constrained ordination technique, was used to explore the relationships among the distributions of diatom taxa and the 19 measured environmental variables (Tables 1, 2). CCA was also used to identify a subset of environmental variables that explained both significant ($p \leq 0.05$) and partially independent directions of variation in the diatom data. To do this, a series of CCAs were run to assess colinear environmental variables (i.e., variables with variance inflation factors (VIF) > 20). The variable with the highest VIF was omitted from the environmental matrix, and the CCA was rerun until all environmental VIFs were <20. Canonical coefficients and approximate *t* tests were then used to identify the subset of variables that each explained significant directions of variance in the distribution of the diatom taxa (Ter Braak

Table 2. Water-chemistry characteristics of the 54 study lakes used as possible explanatory variables for the distributions of surface-sediment diatom assemblages.

Lake No.	pH	Alkal-inity	Cond-uctivity	[DOC]	[Ca]	[Mg]	[Na]	[K]	[SO_4]	[NH_3]	[NO_3]	[TN]	[Si]	[TP]
1	7.04	9.57	53.33	3.30	5.90	1.45	1.90	0.73	8.83	11.00	104.25	220.00	1.03	4.73
2	7.07	11.89	66.50	6.40	5.35	1.26	5.10	0.82	5.24	30.00	2.00	400.00	1.66	21.50
3	6.28	4.27	32.20	4.40	2.48	0.59	2.35	0.41	4.57	54.00	40.00	280.00	0.70	18.60
4	6.68	7.56	39.48	4.21	4.18	1.10	0.68	0.56	7.51	12.09	85.73	277.64	0.68	6.55
5	6.31	2.85	28.73	3.35	2.55	0.71	0.77	0.41	6.77	9.18	61.37	223.87	0.49	5.42
6	6.69	4.49	28.39	1.78	2.55	0.75	0.79	0.38	6.23	21.88	16.57	182.35	0.13	4.97
7	6.93	9.13	51.00	3.17	5.70	1.40	1.70	0.73	8.77	9.75	122.75	226.67	0.99	5.63
8	6.79	5.17	38.70	4.00	3.55	1.00	1.68	0.71	6.19	11.00	2.00	280.00	0.16	14.90
9	6.54	4.46	30.38	2.93	2.86	0.79	0.75	0.49	6.66	18.13	122.75	240.00	0.45	7.19
10	7.03	11.04	82.80	5.80	4.95	1.09	9.01	0.85	4.20	11.00	2.00	360.00	0.60	18.10
11	7.30	21.90	87.00	4.35	14.20	2.30	3.60	0.95	9.10	12.00	170.00	290.00	1.50	8.95
12	5.65	1.00	28.34	4.92	2.43	0.70	0.73	0.33	7.79	21.46	62.40	272.23	1.15	8.31
13	5.79	0.42	25.21	1.69	2.18	0.54	0.49	0.35	7.73	14.90	16.12	156.89	0.11	3.91
14	5.61	0.82	26.69	4.47	2.28	0.63	0.65	0.31	7.46	23.44	54.49	299.33	0.66	10.79
15	5.88	1.31	33.23	5.20	2.69	0.68	1.45	0.38	7.27	31.07	42.57	293.70	0.52	7.50
16	7.17	12.98	56.00	3.37	6.30	1.72	1.07	0.75	8.73	12.00	101.25	210.00	1.04	6.35
17	6.70	3.43	51.90	2.00	3.61	0.80	3.88	0.58	8.05	8.00	231.00	160.00	0.32	3.15
18	7.16	9.91	79.56	3.93	5.68	1.24	6.25	0.90	8.67	29.31	169.62	326.15	0.62	12.48
19	7.19	14.85	66.50	3.80	7.55	2.00	2.18	0.83	9.35	11.67	166.33	240.00	1.51	9.45
20	5.83	1.72	28.72	5.03	2.51	0.71	0.75	0.38	7.59	54.92	20.39	344.74	1.36	8.13
21	7.03	10.71	50.00	3.47	5.40	1.33	0.92	0.77	7.97	7.50	120.25	206.67	1.06	7.63
22	6.47	2.83	35.00	2.40	3.13	0.73	0.92	0.52	8.33	9.25	132.00	146.67	0.73	3.55
23	6.61	3.67	52.60	1.90	3.61	0.80	3.84	0.58	8.00	5.00	247.00	140.00	0.34	3.10
24	7.16	12.58	64.20	4.90	5.84	1.27	4.48	0.78	5.77	47.00	31.00	380.00	1.52	24.30
25	6.36	3.74	37.43	3.68	2.94	0.94	1.60	0.55	7.44	10.21	97.26	220.52	1.09	6.33
26	5.77	0.47	24.27	3.22	1.82	0.52	0.83	0.37	6.60	17.22	23.62	239.33	0.37	5.58
27	7.20	20.80	84.00	4.45	13.50	2.30	3.45	0.95	9.00	12.00	210.00	310.00	1.53	6.65
28	7.21	22.99	83.00	4.50	14.10	2.15	3.30	0.90	8.70	14.00	230.00	260.00	1.87	9.53
29	6.20	1.71	26.33	2.30	2.75	0.63	0.63	0.48	7.20	21.00	85.33	155.00	0.78	5.03
30	6.32	2.01	30.33	2.80	2.83	0.72	0.58	0.53	7.73	24.25	155.00	166.67	0.87	2.70
31	6.45	3.14	41.00	3.13	3.07	0.75	2.60	0.45	6.87	11.75	122.00	170.00	1.26	5.35
32	6.54	4.08	44.33	3.27	3.63	0.75	2.73	0.48	7.10	18.00	117.50	190.00	1.14	6.05
33	6.82	5.74	37.46	2.77	3.10	0.90	1.09	0.63	7.07	33.70	82.80	242.00	1.09	10.53
34	6.31	2.55	31.67	3.90	3.00	0.75	0.60	0.55	7.33	17.25	191.50	213.33	1.59	5.87
35	6.10	1.74	31.00	3.67	3.00	0.72	0.58	0.48	7.97	21.75	224.50	206.67	1.39	5.12
36	6.22	2.51	29.50	4.27	2.77	0.68	0.78	0.44	7.37	34.49	46.71	290.23	0.39	8.30
37	6.04	1.11	35.65	3.03	2.35	0.59	2.39	0.38	6.91	19.63	33.98	222.23	0.21	5.68
38	7.08	11.45	60.00	3.45	6.35	1.75	1.98	0.78	9.05	14.33	169.67	245.00	1.61	7.25
39	6.27	2.61	33.52	5.25	3.14	0.72	1.10	0.45	7.45	24.36	27.98	338.80	0.56	10.37
40	7.24	15.77	59.67	3.30	7.13	1.92	1.07	0.82	8.47	12.00	101.00	216.67	1.00	6.62
41	7.12	9.02	71.95	3.94	5.15	1.18	5.55	0.81	8.34	25.69	121.64	305.45	0.74	10.30
42	7.11	10.76	49.00	3.50	5.53	1.37	1.07	0.78	8.03	14.50	98.50	236.67	1.07	6.85
43	5.72	0.40	22.17	2.12	1.89	0.47	0.52	0.21	6.57	17.43	16.43	174.18	0.37	4.81
44	6.46	4.64	31.56	2.96	2.79	0.87	0.78	0.40	7.03	83.86	31.03	285.33	1.34	5.20
45	6.32	3.23	29.41	2.61	2.57	0.81	0.76	0.39	7.01	12.62	104.65	181.01	1.15	4.21
46	6.33	2.09	29.33	2.60	2.85	0.70	0.45	0.53	7.65	24.33	102.33	185.00	0.76	4.38
47	6.47	2.92	33.33	2.95	2.95	0.78	0.68	0.54	7.95	18.20	151.20	175.00	1.22	5.00
48	6.75	3.36	35.46	2.72	2.99	0.91	1.39	0.47	7.29	9.00	155.20	168.00	1.60	3.91
49	6.71	3.40	35.44	3.06	2.96	0.89	1.43	0.45	7.30	9.80	151.80	172.00	1.62	4.33
50	6.65	3.72	29.12	2.12	2.63	0.72	0.74	0.51	6.84	14.57	74.56	208.33	0.13	4.59
51	7.00	13.20	64.00	5.45	8.90	2.20	2.50	0.80	9.30	10.00	210.00	300.00	1.71	6.18
52	6.34	2.46	35.33	2.97	2.80	0.68	1.90	0.37	6.77	13.00	112.00	166.67	1.13	3.75
53	6.67	4.58	41.07	3.37	3.18	0.94	1.89	0.66	6.91	51.15	57.66	281.64	0.82	5.40
54	6.69	5.08	30.59	3.12	2.81	0.75	0.93	0.55	6.33	13.13	65.19	237.08	0.44	8.18
Min.	5.61	0.40	22.17	1.69	1.82	0.47	0.45	0.21	4.20	5.00	2.00	140.00	0.11	2.70
Max.	7.30	22.99	87.00	6.40	14.20	2.30	9.01	0.95	9.35	83.86	247.00	400.00	1.87	24.30
Mean	6.58	6.29	43.77	3.54	4.31	1.03	1.88	0.58	7.45	20.35	101.76	239.25	0.93	7.58
SD	0.47	5.63	17.91	1.06	2.84	0.49	1.70	0.19	1.10	14.59	67.01	63.59	0.48	4.51

Note: Total phosphorus ([TP]) concentrations represent spring overturn values. Values for all other variables are volume-weighted ice-free concentrations. [NH_3] included ammonia plus ammonium; [NO_3] included nitrate plus nitrite; [TN] was measured as total Kjeldahl nitrogen (Janhurst 1994). Summary statistics (minimum, maximum, mean, and standard deviation (SD)) are presented at the bottom of the table. Lake names are presented in Table 1. Data are given in the following units: alkalinity, $mg \cdot L^{-1}$, as $CaCO_3$; conductivity, $\mu S \cdot cm^{-1}$; [DOC], [Ca], [Mg], [Na], [K], [SO_4], [NO_3], [TN], and [Si], $mg \cdot L^{-1}$; [NH3] and [TP], $\mu g \cdot L^{-1}$.

Table 3. Canonical coefficients, approximate *t* test values, and intraset correlations of the environmental variables for each of the first three CCA axes.

	Canonical coefficients			*t* values			Intraset correlations		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
pH	1.05	0.04	0.59	–14.7*	0.3	3.2*	–0.94	0.26	0.15
[DOC]	0.04	0.45	0.09	0.5	3.3*	0.4	0.13	0.82	0.20
[Na]	–0.02	–0.43	–0.09	–0.4	–3.9*	–0.5	–0.34	0.15	0.31
[SO_4]	0.11	0.28	–0.79	1.6	2.4*	–4.5*	–0.40	0.14	–0.82
[NH_3]	0.00	–0.14	0.45	0.0	–1.5	3.1*	0.34	0.21	0.54
[Si]	–0.01	0.44	–0.28	–0.1	3.8*	–1.6	–0.34	0.57	–0.19
[TP]	0.34	0.36	0.09	3.5*	2.2*	0.4	0.05	0.59	0.65
Z_{max}	–0.08	–0.49	0.21	–4.9*	–4.3*	1.2	–0.26	–0.44	–0.38

*Significant at $p \leq 0.05$.

1987*a*). In all CCAs, taxon scores were scaled to be weighted averages of the site scores (Ter Braak 1990). The significance of the CCA axes was assessed using Monte Carlo permutation tests (999 random permutations; Ter Braak 1988*a*).

Weighted-averaging regression and calibration (WA) models, with classical deshrinking, were developed using the computer program WACALIB version 3.3 (Line et al. 1994). Errors associated with model inferences were estimated from 1000 bootstrap cycles (Birks et al. 1990*b*). WA inference models were assessed using (*i*) the correlation between measured and bootstrapped diatom-inferred values, (*ii*) the apparent root mean squared error of prediction (RMSE), and (*iii*) the bootstrap RMSE (Birks et al. 1990*a*).

Water-chemistry inferences from diatoms are likely to be more reliable if fossil assemblages have close analogues within the modern calibration samples (Birks et al. 1990*b*). We used a method of analogue matching (developed by Birks et al. 1990*b*) to identify preindustrial diatom assemblages with poor analogy to the modern assemblages. Briefly, a squared χ^2 dissimilarity index (Prentice 1980) was used to compare every fossil sample with all calibration samples. Fossil samples with a minimum squared χ^2 greater than the extreme 5% of the squared χ^2 distributions for comparisons among all modern calibration samples were considered to lack a good modern analogue. Analogue matching was computed using the computer program ANALOG version 1.6 (J.M. Line (Cambridge University) and H.J.B. Birks (University of Bergen), unpublished program).

CCA ordinations and WA regression and calibration were performed using both untransformed environmental variables, some of which had skewed distributions, and data transformed to normality.

Quality assurance – quality control

Intercore variability, intracore variability, and taxonomic inconsistency are factors that potentially influence the reliability of inferences from sedimentary diatoms. In general, the variability associated with sedimentary diatom sample preparation (subsampling, processing, and counting) is relatively small (Charles et al. 1991). In this study, triplicate cores were collected from the deepwater regions of three lakes (Maple, Dickie, and Bass) to test the reliability of our techniques to infer present-day and preindustrial water quality.

Results and discussion

Ordination

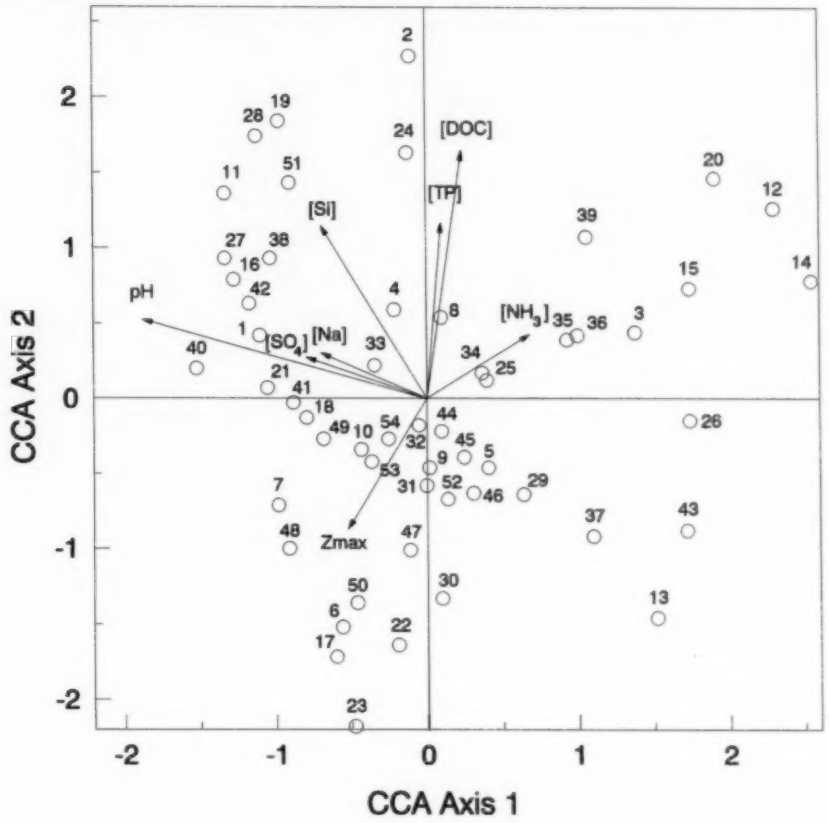
Eigenvalues ($\lambda_1 = 0.37$, $\lambda_2 = 0.22$) of the first two DCA axes captured 27.6% of the cumulative variation in the diatom data. The low percentage of explained variance is typical of noisy data sets containing a large number of taxa and many zero values (Stevenson et al. 1991). Axes 1 and 2 included species gradients with lengths of 3.0 and 2.3 standard deviation units, respectively, suggesting that numerical techniques based on a unimodal species model were most appropriate (Ter Braak and Prentice 1988).

Transforming the environmental data to normality had very little, if any, effect on CCA ordinations (data not shown), and so we chose to present ordination results based on the untransformed data.

After deleting colinear environmental variables (i.e., those with VIFs > 20; see Methods), CCA identified eight environmental variables (pH, [DOC], [Na], [SO_4], [NH_3], [Si], [TP], and maximum depth (Z_{max})) that each explained significant ($p \leq 0.05$) directions of variance in the diatom data along one or more of the first three axes (Table 3). The eigenvalues for CCA axes 1 (0.33), 2 (0.21), and 3 (0.12) constrained to these eight environmental variables were significant ($p \leq 0.01$), and they were similar to those for an unconstrained DCA of the diatom data (0.37, 0.22, 0.10). The species–environmental correlations for CCA axes 1 (0.96), 2 (0.89), and 3 (0.78) were high. The eight environmental variables, therefore, accounted for the major gradients in the composition of diatom assemblages within this lake set. They explained 69% of the variance captured by the original 19 variables.

On the basis of the canonical coefficients of environmental variables and approximate *t* tests (Ter Braak 1987*b*, 1988*a*), and by their intraset correlations with the axes (Ter Braak 1987*b*), CCA axis 1 captured a gradient of pH ($r = -0.94$) (Table 3). Axis 2 was highly correlated with lake trophic status, [DOC], and physical lake characteristics. [DOC] ($r = 0.82$), [Si] (0.57), [TP] (0.59), and maximum lake depth (–0.44) all contributed significantly to CCA axis 2 (Table 3). The influences of [TP] and lake depth were split along axis 2, indicating that CCA was able to separate diatom assemblages that occur in relatively shallow water, nutrient-rich lakes, from those occurring in deeper, more oligotrophic lakes. Lake water

Fig. 1. Canonical correspondence analysis (CCA) ordination biplot of the 54-lake set. Lakes are represented by open circles. Lake names are presented in Table 1. Arrows indicate environmental variables that are significantly correlated with the distributions of diatoms. Environmental variable biplot scores are multiplied by a factor of two.



[TP] and [DOC] accounted for similar directions of variance in the diatom data (Table 3, Figs. 1, 2), probably because the majority of bioavailable phosphorus is associated with organic compounds in these lakes (P. Dillon, OMOEE, Dorset Research Centre, Dorset, Ont., personal communication). CCA axis 3 captured variance attributable mainly to $[SO_4]$ (r = –0.82) and $[NH_3]$ (0.54).

In CCA biplots (Fig. 1), axis 1 effectively separates acidic lakes (e.g., Chub (No. 12), Crosson (14), Dickie (15), Heney (26), and Plastic (43); positioned along the right side) from the more alkaline lakes (e.g., Haliburton (21), Moose (40), and Oblong (42); positioned along the left side). Diatom taxa that were most abundant in acidic lakes are likewise positioned along the right side of the CCA biplot (e.g., *Asterionella ralfsii* var. *americana* (No. 19), *Eunotia paludosa* (42), and *Frustulia magaliesmontana* (57)), whereas taxa more characteristic of circumneutral or alkaline waters are positioned along the left side (e.g., *Fragilaria construens* (46), *Synedra cyclopum* (82)) (Fig. 2). Axis 2 reflects a gradient of lake trophic status and distinguishes diatom taxa characteristic of shallow lakes with relatively high [TP] and [DOC] (positioned towards the top of the biplot; e.g., *Aulacoseira ambigua* (21), *Aulacoseira subarctica* (25)) from those characteristic of deeper, oligotrophic, clear-water lakes (positioned at the bottom of the biplot; e.g., *Cyclotella kuetzingiana* (29), *Cyclotella ocellata* (31)) (Fig. 2).

In summary, CCA ordinations suggest that there are statistically significant relationships between diatom distributions and the eight environmental variables (pH, [DOC], [Na], $[SO_4]$, $[NH_3]$, [Si], [TP], and Z_{max}). These variables can, therefore, potentially be inferred from fossil diatom assemblages in our calibration set.

We now focus on developing inference models for hindcasting trends in lake water pH and [TP], as these are the best variables for assessing water-quality changes resulting from atmospheric deposition and lakeshore cottage development in Precambrian Shield lakes (e.g., Neary et al. 1990; Dillon et al. 1993).

Inference models for hindcasting lake water pH and [TP]

Reliable water-chemistry inferences from sedimentary diatom assemblages require a sufficiently strong statistical relationship between diatoms and the variable to be reconstructed (Ter Braak 1987*a*). To develop quantitative inference models, the

Fig. 2. Canonical correspondence analysis (CCA) ordination biplot of the 54-lake set. Diatom taxa are represented by closed circles. Diatom names are presented in Table 5. Arrows indicate environmental variables that are significantly correlated with the distributions of diatoms.

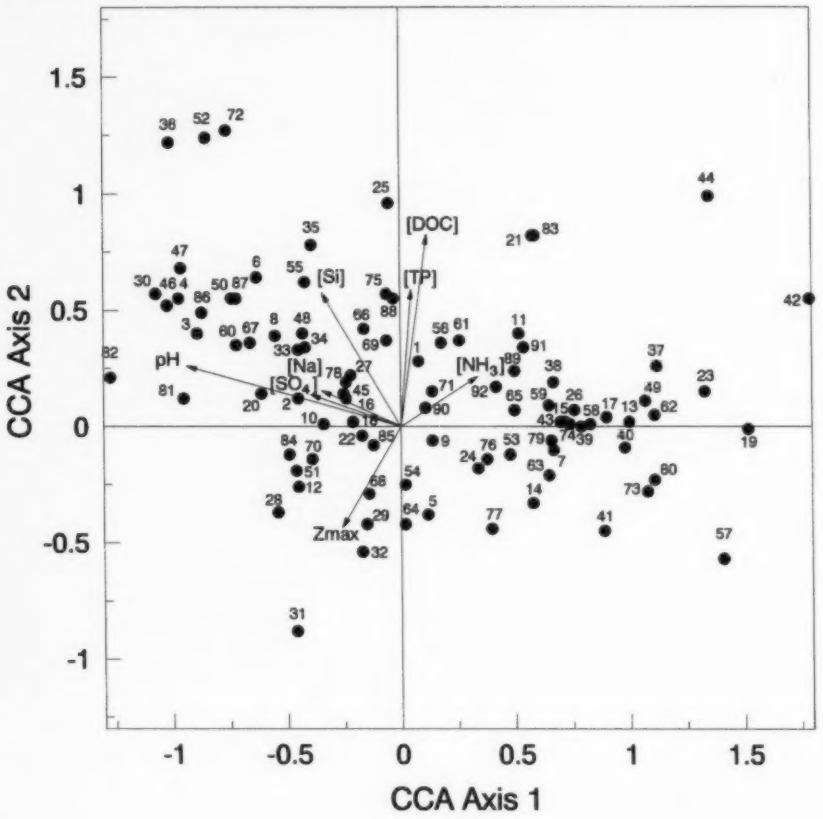


Table 4. Constrained and partially constrained CCA results indicating the strength and partial independence of pH and [TP].

	Constrained CCA			Partially constrained CCA		
Variable	λ_1	λ_1/λ_2	p	λ_1	λ_1/λ_2	p
pH	0.31	1.12	0.01	0.18	1.10	0.01
TP	0.13	0.36	0.01	0.05	0.29	0.06

Note: λ_1 and λ_2 are eigenvalues for axis 1 and 2, respectively. p values are based on 99 unrestricted Monte Carlo permutations.

strengths of the relationships between pH, [TP], and diatoms were assessed in two ways: using constrained CCAs and partially constrained CCAs (Ter Braak 1988*b*).

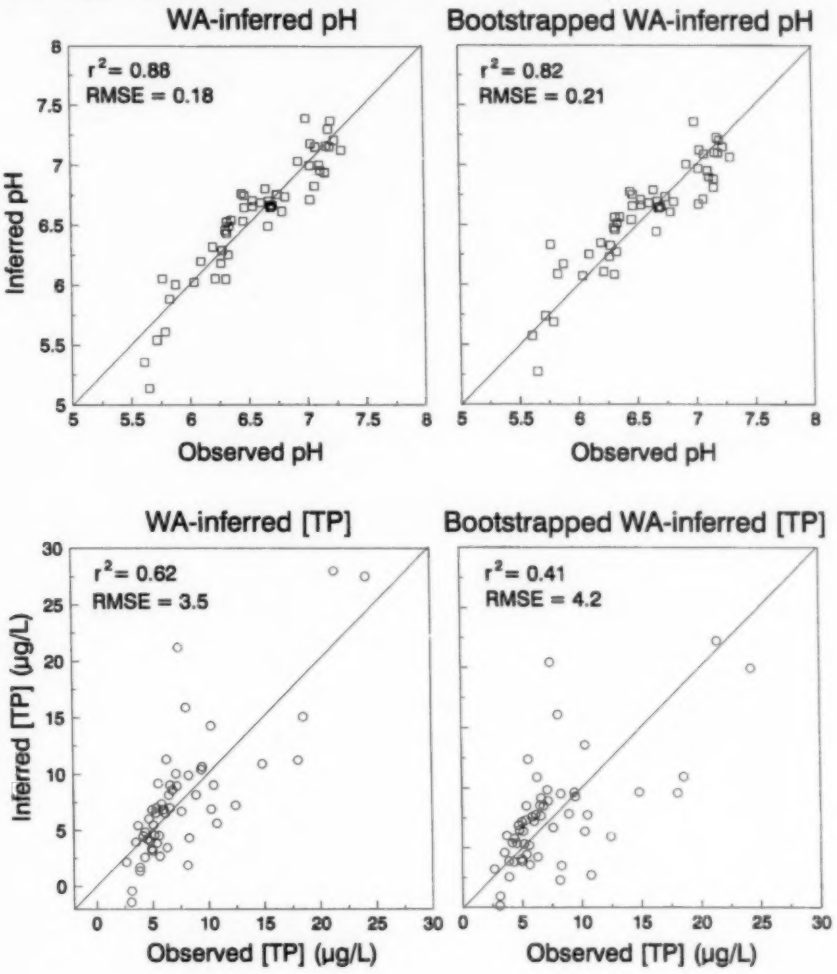
In the first step, constrained CCAs were each run with a single environmental variable (Ter Braak 1988*b*). In this way, species composition was constrained to one external variable. Reliable inference models can be developed for environmental variables with a high ratio of the first (constrained) eigenvalue (λ_1) to the second (unconstrained) eigenvalue (λ_2) (Ter Braak 1988*a*).

Of all the environmental variables, pH was most strongly correlated with diatom assemblages in Muskoka-Haliburton lakes, and pH had the highest ratio of λ_1/λ_2 ($\lambda_1/\lambda_2 = 1.1$; Table 4). Other studies have demonstrated similar correlations between diatom assemblages and pH (e.g., Dixit et al. 1991) and have documented the direct influence of pH on diatoms (Gensemer 1991). The ratio of λ_1/λ_2 for [TP] was 0.36 (Table 4), indicating that [TP] was an important variable influencing diatom distributions, but that other factors also controlled diatom assemblage composition.

In the second step, partially constrained CCAs and unrestricted Monte Carlo permutation tests were used to examine the individual effect of each environmental variable on the distribution of diatom taxa. In this way, the first ordination axis was constrained to a single environmental variable after the influences of the other seven variables were partialled out (Ter Braak 1988*b*). This analysis indicated significant ($p \leq 0.05$) and independent responses of diatoms to pH, but not to [TP] (Table 4).

Diatom responses to [TP] were independent of pH, [Si], [NH_3], and lake depth, but covaried with [DOC] (data not shown). The covariance of diatoms along [TP] and [DOC]

Fig. 3. The relationship between measured and diatom-inferred lake water pH and total phosphorus concentration ([TP]) using weighted-averaging regression and calibration models and classical deshrinking, with and without bootstrapping.



gradients was not surprising, because lakes with the highest [TP] in this calibration set also had the highest [DOC]. Furthermore, the majority of bioavailable phosphorus is associated with dissolved organic compounds in Muskoka-Haliburton lakes (P. Dillon, OMOEE, Dorset Research Centre, Dorset, Ont., personal communication).

In our lake set, λ_1/λ_2 values for pH and [TP] (Table 4) were comparable to those for WA inference models developed in other regions of North America (e.g., Dixit et al. 1991, 1993 (pH); Cumming et al. 1992 (pH); Hall and Smol 1992 (TP); Fritz et al. 1993 (TP); Reavie et al. 1995 (TP)). We therefore conclude that the relationship is sufficiently robust for developing pH and [TP] inference models based on WA techniques. However, [TP] inferences should also be interpreted with the consideration that [DOC] and other unmeasured variables may strongly influence diatom responses to [TP].

To develop robust and reliable WA inference models, realistic optima of diatom taxa must be estimated along the environmental gradients of interest. One way to assess whether estimates of diatom optima are ecologically realistic is to compare goodness of fit (i.e., r^2 and RMSE of prediction) between measured versus WA-inferred values estimated with and without bootstrapping. If the goodness-of-fit between measured and inferred values is much weaker when estimated using bootstrapping, then species optima are not clearly defined by WA techniques.

Our 54-lake calibration set reliably estimated the optima of diatom taxa along the pH gradient, as indicated by relatively small differences between correlations (0.82 vs. 0.88) and root mean squared errors (0.21 vs. 0.18) derived from weighted-averaging models constructed with and without bootstrapping (Fig. 3). For [TP], however, the relationships between

Table 5. The optima and effective number of occurences (Hill's N2) of the diatom taxa used in ordinations and WA regression and calibration models.

Code	Taxon	pH	[TP] ($\mu g \cdot L^{-1}$)	N2
1	*Achnanthes bioreti* Germ.	6.54	6.94	13.7
2	*Achnanthes chlidanos* Hohn & Hellermann	6.74	5.74	4.8
3	*Achnanthes flexella* Kütz. (Brun)	6.97	6.59	7.8
4	*Achnanthes laevis* Østrup	7.02	6.98	7.4
5	*Achnanthes levanderi* Hustedt	6.48	6.40	15.5
6	*Achnanthes linearis* (W. Smith) Grun. sensu auct. nonnull.	6.95	9.56	11.4
7	*Achnanthes marginulata* Grun.	6.28	7.42	8.7
8	*Achnanthes minutissima* Kütz.	6.85	7.57	26.6
9	*Achnanthes subatomoides* (Hust.) Lange-Bertalot & Archibald	6.53	8.13	21.6
10	*Aulacoseira distans* var. *humilis* (Cleve-Euler) Simonsen	6.74	7.83	5.3
11	*Aulacoseira distans* var. *tenella* (Nygaard) (Florin) Simonsen	6.36	8.09	12.5
12	*Aulacoseira distans* var. 3 PIRLA	6.64	4.89	3.7
13	*Aulacoseira lirata* (Ehr.) Ross	6.18	8.71	14.9
14	*Aulacoseira lacustris* (Grun.) Krammer	6.26	5.64	4.3
15	*Anomoeoneis brachysira* (Bréb) Grun. (in Cleve)	6.26	7.34	19.5
16	*Anomoeoneis vitrea* (Grun.) Ross	6.66	6.71	27.6
17	*Aulacoseira perglabra* var. *fluoriniae* (Camburn) Simonsen	6.20	7.86	14.4
18	*Asterionella formosa* Hassall	6.68	7.92	33.9
19	*Asterionella ralfsii* var. *americana* Körner	5.90	6.70	9.5
20	*Achnanthes suchlandtii* Hust.	6.85	7.37	6.9
21	*Aulacoseira ambigua* (Grun.) Simonsen	6.49	11.78	12.7
22	*Aulacoseira distans* (Ehr.) Simonsen	6.61	6.61	22.5
23	*Aulacoseira nygaardii* (Camburn) Simonsen	6.07	9.57	3.9
24	*Aulacoseira perglabra* (Østrup) Haworth	6.42	7.60	6.4
25	*Aulacoseira subarctica* (O. Müll.) Haworth	6.84	14.53	10.0
26	*Aulacoseira* cf. sp. 1 PIRLA	6.30	9.01	6.9
27	*Cyclotella bodanica* var. *lemanica* (O. Müll.) Bachmann	6.70	7.83	26.5
28	*Cyclotella kuetzingiana* var. *radiosa* sensu Fricke	6.74	5.22	16.6
29	*Cyclotella kuetzingiana* sensu Thwaites	6.55	5.20	9.2
30	*Cyclotella michiganiana* Skvortzow	7.08	7.26	10.7
31	*Cyclotella ocellata* Pantocsek	6.66	4.55	11.2
32	*Cyclotella stelligera* Cleve & Grun. (in Van Heurck)	6.61	6.49	33.4
33	*Cymbella cesatii* (Rabenh.) Grun.	6.79	7.22	10.7
34	*Cymbella microcephala* Grun. (in Van Heurck)	6.78	7.39	18.5
35	*Cymbella silesiaca* Bleisch	6.86	10.09	16.4
36	*Diatoma tenue* var. *elongatum* Lyngb.	7.13	8.05	2.8
37	*Eunotia bilunaris* var. *mucophila* Lange-Bertalot & Nörpel	6.19	11.11	3.6
38	*Eunotia pectinalis* var. *minor* (Kütz.) Rabenh.	6.33	8.20	7.4
39	*Eunotia bilunaris* (Ehr.) Mills	6.21	6.53	8.8
40	*Eunotia exigua* (Bréb.) Rabenh.	6.10	6.04	6.3
41	*Eunotia meisteri* Hust.	6.11	5.50	5.3
42	*Eunotia paludosa* Grun.	5.86	8.86	2.8
43	*Eunotia rhomboidea* Hust.	6.27	7.65	7.2
44	*Eunotia zasuminensis* (Cab.) Körner	6.09	9.57	2.8
45	*Fragilaria brevistriata* Grun.	6.71	8.34	26.1
46	*Fragilaria construens* (Ehr.) Grun.	7.05	6.70	6.8
47	*Fragilaria crotonensis* Kitton	7.05	7.64	14.3
48	*Fragilaria pinnata* Ehr.	6.83	9.21	17.1
49	*Fragilaria* sp. 5 PIRLA	6.09	6.69	9.1
50	*Fragilaria vaucheriae* (Kütz.) Petersen	6.95	7.80	11.6
51	*Fragilaria brevistriata* var. *papillosa* Cleve-Euler	6.71	5.79	8.6
52	*Fragilaria capucina* var. *mesolepta* (Rabenh.) Rabenh.	7.16	12.77	4.8
53	*Fragilaria construens* var. *binodis* (Ehr.) Grun.	6.32	6.23	5.4
54	*Fragilaria construens* var. *pumila* Grun.	6.54	7.07	4.4
55	*Fragilaria construens* f. *venter* (Ehr.) Hust.	6.79	7.27	13.4
56	*Fragilaria pinnata* var. *lancettula* (Schumann) Hust.	6.68	13.39	8.2

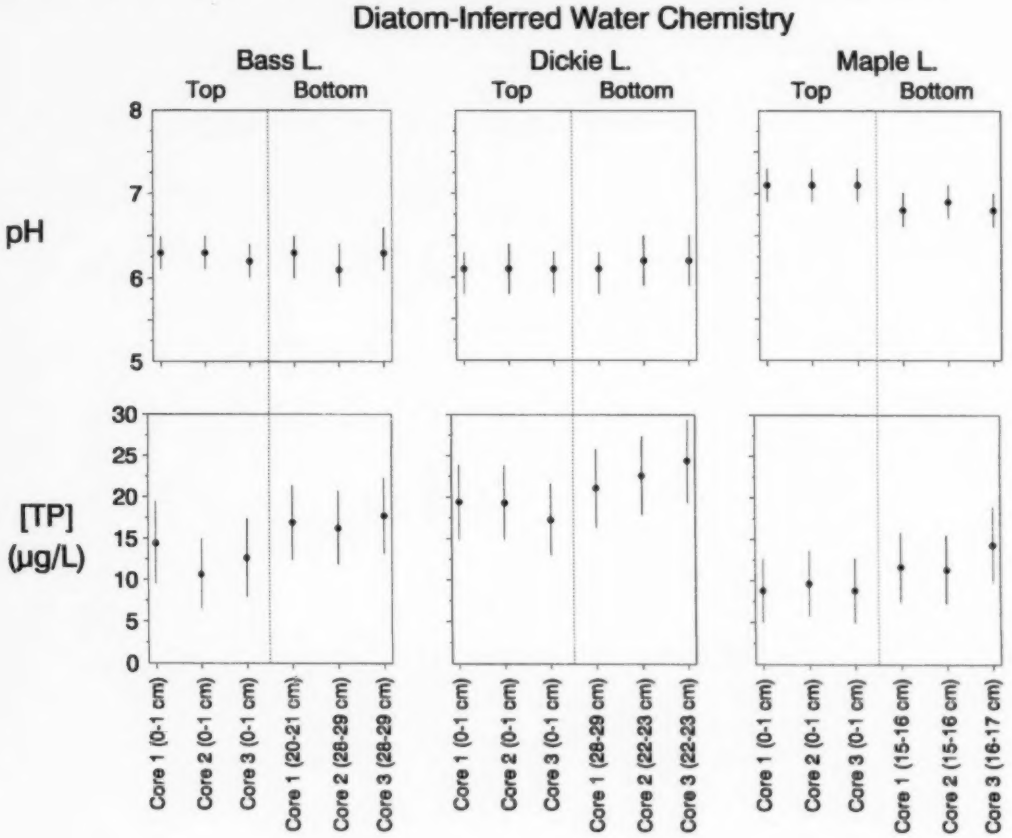
Table 5 (*concluded*).

Code	Taxon	pH	[TP] ($\mu g \cdot L^{-1}$)	N2
57	*Frustulia magaliesmontana* Cholnoky	5.88	5.19	3.4
58	*Frustulia rhomboides* (Ehr.) De Toni	6.22	7.29	14.1
59	*Fragilaria virescens* var. *exigua* Grun.	6.33	8.54	13.2
60	*Gomphonema* cf. *lateripunctatum* Reichardt & Lange-Bertalot	6.87	5.96	3.2
61	*Navicula laevissima* Kütz.	6.60	11.56	6.8
62	*Navicula leptostriata* Jørgensen	6.06	6.36	7.5
63	*Navicula mediocris* Krasske	6.29	7.81	5.7
64	*Navicula pseudoscutiformis* Hust.	6.48	5.49	6.7
65	*Navicula pupula* Kütz.	6.39	8.50	15.6
66	*Navicula pseudoventralis* Hust.	6.76	10.38	6.5
67	*Navicula rhynchocephala* Kütz.	6.87	6.97	7.9
68	*Navicula vitiosa* Schimanski	6.62	7.53	7.1
69	*Navicula cryptocephala* Kütz.	6.68	9.55	21.1
70	*Nitzschia* cf. *fonticola* Grun.	6.71	6.20	7.7
71	*Nitzschia* cf. *gracilis* Hantzsch	6.55	8.20	22.6
72	*Nitzschia* cf. *palea* (Kütz.) W. Smith	7.05	10.17	5.8
73	*Nitzschia* cf. *perminuta* (Grun.) M. Peragallo	6.06	6.11	7.7
74	*Navicula subminiscula* Manguin	6.31	9.38	6.1
75	*Navicula submuralis* Hust.	6.67	8.85	13.2
76	*Pinnularia abaujensis* (Pantoscek) Ross	6.46	9.16	6.7
77	*Pinnularia braunii* (Grun.) Cleve	6.38	7.46	3.3
78	*Rhizoselenia* cf. *eriensis* H.L. Smith	6.74	8.14	12.5
79	*Stauroneis anceps* Ehr.	6.34	9.59	9.0
80	*Stauroneis phoenicenteron* (Nitzsch) Ehr.	6.09	7.70	6.6
81	*Synedra acus* var. *angustissima* Grun.	7.15	10.45	3.0
82	*Synedra cyclopum* Brutschy	7.13	6.24	3.5
83	*Synedra famelica* Kütz.	6.51	12.94	5.5
84	*Synedra filiformis* var. *exilis* Ceve-Euler	6.79	7.17	18.4
85	*Synedra nanana* Meister	6.62	7.12	15.3
86	*Synedra rumpens* Kütz.	7.03	8.99	5.2
87	*Synedra tenera* W. Smith	6.96	8.38	6.2
88	*Tabellaria flocculosa* str. III sensu Koppen	6.63	7.44	10.3
89	*Tabellaria flocculosa* str. IIIp sensu Koppen	6.39	7.99	30.1
90	*Tabellaria flocculosa* var. *linearis* Koppen	6.55	7.05	10.5
91	*Tabellaria flocculosa* str. IV sensu Koppen	6.36	7.47	25.5
92	*Tabellaria quadriseptata* Knudson	6.41	7.02	6.4

bootstrapped WA-inferred and measured values (r^2 = 0.41, RMSE = 3.5 $\mu g \cdot L^{-1}$) were weaker than those between straight WA-inferred and measured values (r^2 = 0.62, $RMSE_{boot}$ = 4.2 $\mu g \cdot L^{1}$) (Fig. 3). Transforming [TP] to normality (log[TP]) did not significantly improve the performance of bootstrapped WA inferences (data not shown). To simplify the presentation of diatom-inferred [TP] trends and their associated error estimates, we chose to present WA models based on untransformed [TP] data. These results indicate that optima of diatoms for [TP] are not as well defined in our calibration set as they are for pH, in part because the [TP] gradient length is shorter than for pH. Also, the relationships between diatoms and [TP] may not be quite so strong because other factors, such as intracellular storage, physical turbulence, interactions with dissolved organic molecules, and light penetration may also strongly influence diatoms (e.g., Reynolds 1980, 1994). As more lakes are added to this data set, the [TP] inference model may become more robust.

In general, the diatom inference models provide close estimates of measured water chemistry (Fig. 3). Standard errors are relatively small and data points are evenly distributed about a 1:1 line (Fig. 3). The pH model is accurate to within ±0.21 pH units, and the [TP] model to within ±4.2 $\mu g \cdot L^{-1}$, on the basis of the $RMSE_{boot}$ (Fig. 3). Our inference models are comparable to models developed in other regions of North America. For example, the correlations and RMSE of prediction for the pH model (r^2 = 0.88, $RMSE_{boot}$ = 0.21) are similar to those for a 71-lake set in the Adirondacks, New York (r^2 = 0.91, $RMSE_{boot}$ = 0.35; Dixit et al. 1993), and a 64-lake set from the northeastern U.S.A. (r^2 = 0.86, RMSE = 0.25; Dixit and Smol 1994). Our [TP] inference model (r^2 = 0.62, $RMSE_{boot}$ = 4.2 $\mu g \cdot L^{-1}$, RMSE = 3.5 $\mu g \cdot L^{-1}$) compares well with models developed from 64 lakes in the northeastern U.S.A. (0.62, ln(TP + 1) RMSE = 0.66 $\mu g \cdot L^{-1}$; Dixit and Smol 1994), a 41-lake data set from Michigan (0.73, ln(TP + 1) RMSE = 0.41 $\mu g \cdot L^{-1}$; Fritz et al. 1993), a 64-lake data set from

Fig. 4. Diatom-inferred pH and total phosphorus concentration ([TP]) from the top (0–1 cm) and bottom (the lowermost 1-cm interval) sediment samples of triplicate deepwater cores. Error bars represent ±$RMSE_{boot}$ (see text for details).



British Columbia (0.69, ln(TP + 1) RMSE = 0.9 $\mu g \cdot L^{-1}$; Reavie et al. 1995), and a 30-lake data set from England (0.79, log[TP] $RMSE_{boot}$ = 0.28 $\mu g \cdot L^{-1}$; Bennion 1994). We therefore conclude that our pH and [TP] inference models are sufficiently robust to allow quantitative hindcasts of these variables from sediment diatom assemblages in south-central Ontario lakes.

To allow interested researchers to use our diatom inference models, we list the pH and [TP] optima of the 92 common diatom taxa and the effective number of occurrences (i.e., analogous to Hill's N2: Hill 1973, 1979; Ter Braak 1990) upon which they are based in Table 5. Classical deshrinking regression equations are as follows for pH and [TP] ($\mu g \cdot L^{-1}$), respectively: $y = 0.315x + 4.51$, and $y = 0.134x + 6.56$.

How reliable are diatom-inferred chemistry values?

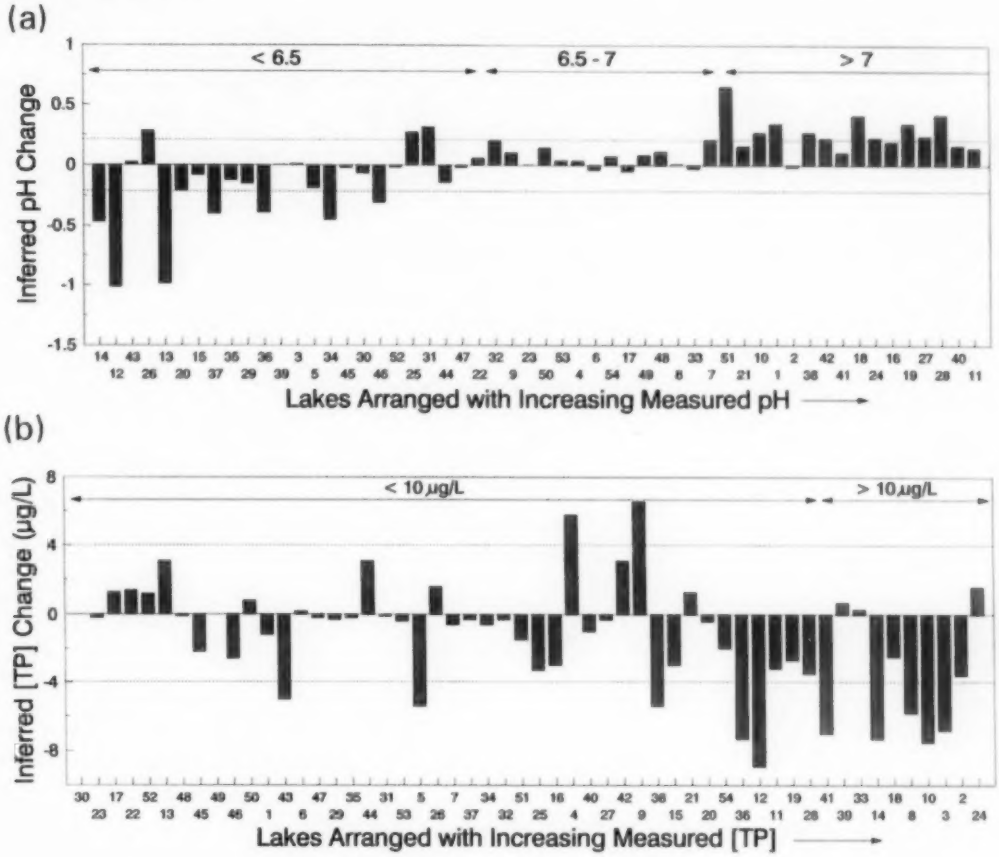
Several lines of evidence suggest that diatom-inferred water chemistry was reliable. First, all fossil diatom assemblages possessed good modern analogues within the calibration set, indicating that water chemistry inferences were well represented. Second, water chemistry inferences from the tops and bottoms of triplicate cores taken from three of the study lakes showed good agreement (Fig. 4). Variability among replicate present-day and preindustrial inferences was low (Fig. 4), indicating that water chemistry inferences are reproducible from deepwater sediment cores. Third, several large, multi-institution paleoecological research projects have also shown that variability between diatom assemblages in sediment cores collected from the deepwater regions of lakes is relatively small (e.g., Cumming et al. 1990; Davis et al. 1990; Charles et al. 1991). Together, these analyses suggest that single deepwater sediment cores are adequate for tracking community changes and inferring water chemistry trends.

Lake water quality changes since preindustrial times

In general, our paleolimnological evidence suggests that water quality changes were relatively small within the Muskoka-Haliburton lake set (Fig. 5). Within the detection limits of our models, 61% of the lakes showed no significant changes in diatom-inferred pH since preindustrial times and 78% showed no significant [TP] changes.

Diatoms inferred significant pH declines (i.e., greater than the $RMSE_{boot}$) in 13% of the lakes and significant pH increases in 24% of the lakes (Fig. 5*a*). Lakes that are presently acidic

Fig. 5. Diatom-based inferences of historical change (preindustrial to present) in (*a*) pH and (*b*) total phosphorus concentration ([TP]) from the 54 study lakes. Water chemistry changes are presented as the difference between values inferred from diatoms in the top (0–1 cm) and bottom (lowermost 1-cm) sediment core intervals. Broken lines represent $\pm RMSE_{boot}$ of prediction.



have tended to acidify since preindustrial times. For example, more than half (62.5%) of the presently acidic lakes (pH ≤ 6) have had significant diatom-inferred pH declines. Several of these lakes have acidified by between 0.5 and 1 pH units (Fig. 5*a*). In contrast, diatom-inferred pH tended to increase in the more alkaline lakes. Of the lakes with present-day pH ≥ 7, 62.5% had significant increases in diatom-inferred pH. Diatom-inferred pH showed no significant change in presently circumneutral lakes (i.e., pH = 6.5–7) (Fig. 5*a*).

Similar pH trends have been observed at Sudbury, Ontario (Dixit et al. 1992*c*), and in other regions of the northeastern U.S.A. receiving high levels of atmospheric deposition (Cumming et al. 1992; Dixit and Smol 1994). Our paleolimnological evidence, therefore, suggests that water quality in south-central Ontario has been altered by acidic deposition. These results are consistent with the findings of water chemistry monitoring programs, under way since 1979, which have demonstrated a strong correlation between lake water pH declines and sulphate deposition in central Ontario (Neary et al. 1990). The majority of acidified Ontario lakes are located in central Ontario, including Muskoka-Haliburton districts, where total annual sulphur deposition exceeds 0.75 $g \cdot cm^{-2} \cdot yr^{-1}$ (Neary et al. 1990). Natural organic acids are not a major factor explaining the acidification of lakes in this region (Neary et al. 1990). Because we did not sample the extremely acidic lakes (i.e., those with pH < 5.5), our data probably underestimate the full extent of lake acidification in south-central Ontario.

Our diatom-inferred pH data (Fig. 5*a*), long-term monitoring and paleolimnological data from Sudbury (Gunn and Keller 1990; Dixit et al. 1992*b*, 1992*c*), and modelling data from the Adirondacks (Sullivan et al. 1990) all indicate that the pH of alkaline lakes has increased in areas receiving elevated acidic deposition. At least two factors may be responsible for these inferred pH increases, including (*i*) watershed disturbances that increase ANC (Haines and Akielaszek 1983; Eilers et al. 1989) and (*ii*) biogeochemical ANC-generating reactions, such as sulphate reductions by bacteria (Kilham 1982; Cook et al. 1986) and assimilation of atmospherically deposited nitrogen compounds by plants (Sullivan 1990). Our top and bottom sediment survey cannot identify the timing of

these pH increases; however, analysis of sediment intervals deposited during the past 10–100 years from several of the more alkaline lakes will soon be initiated, and we hope to gain a better understanding of the timing and rate of these limnological changes.

In general, diatoms inferred relatively small changes in lake water [TP] since preindustrial times, despite moderate shoreline development, including mainly cottages and past logging, around many of the lakes (Fig. 5*b*). Only two lakes (Basshaunt (No. 4) and Buck (9); Fig. 5*b*) showed significant increases in [TP], and they are presently oligotrophic ([TP] < 10 $\mu g \cdot L^{-1}$). Diatoms may be tracking elevated P loadings resulting from recent cottage developments and small-scale logging activity around these two lakes. However, these activities are not unique to Buck and Basshaunt lakes, and similar disturbances are not reflected by diatom-inferred [TP] changes in other lakes. Perhaps these lakes are more sensitive to watershed activities that elevate P loads than other lakes in this data set, because they have relatively small lake area and volume (e.g., Dillon et al. 1993; Hall and Smol 1993). However, it is also possible that changes in watershed and (or) wetland processes may have enhanced DOC and P loads over natural levels.

We were initially surprised that diatoms inferred no significant increases in lake water [TP] in Gravenhurst Bay (No. 18) since preindustrial times (Fig. 5*b*). Gravenhurst Bay has received the greatest amount of human activity of all the lakes in this study, including nutrient-rich sewage water inputs and industrial activity from the town of Gravenhurst. Prior to 1972, TP concentrations were high (40–50 $\mu g \cdot L^{-1}$) and Gravenhurst Bay often had severe algal blooms, poor water clarity, and hypolimnetic anoxia (Dillon et al. 1978). However, since the installation of improved P removal technologies from sewage waste water in 1972, [TP] steadily declined to 12.5 $\mu g \cdot L^{-1}$ in 1992. Sediment diatom assemblages suggest that lake water [TP] has returned to concentrations similar to those that existed naturally in Gravenhurst Bay.

Diatoms inferred [TP] declines in half of the lakes with relatively high (10–24 $\mu g \cdot L^{-1}$) present-day [TP] in our lake set (Fig. 5*b*), indicating that presently mesotrophic lakes tended to have higher natural [TP] prior to European settlement. None of the currently mesotrophic lakes showed significant increases in diatom-inferred [TP]. The exact reasons for declining [TP] in these lakes are unknown, but several possibilities exist. In several cases, [TP] declines may have been, at least partially, caused by lake and (or) watershed acidification processes that retain P within catchments or that increase the rate of P loss from lakes (e.g., the formation and enhanced precipitation of P-Al compounds: Jansson et al. 1986; Olsson and Pettersson 1993). For example, three of the lakes (Chub (No. 12), Crosson (14), and Leech (36); Fig. 5) with the largest diatom-inferred [TP] declines (9, 7, and 7 $\mu g \cdot L^{-1}$, respectively) also had the greatest inferred pH declines, on the order of 0.4 to 1.0 pH units (Fig. 5). In all cases where inferred lake water pH declined significantly since before 1850, diatom-inferred [TP] never increased, suggesting at least some relationship between pH and [TP] trends within this lake set.

Forest dynamics may also contribute to declining [TP]. According to such studies as those by Likens and Bormann (1974, 1979), the mature, old-growth forest that existed prior to European settlement may have supplied greater P loads to lakes than the younger (50 to 100 year old), logged, and aggrading forests that surround the lakes today. However, many other anthropogenic changes have taken place that could also reduce lake water [TP], including (*i*) reduction in forest fires, (*ii*) alterations in aquatic food-web structure as a result of increases in fish stocking, fish harvesting (angling), and other lake-management activities, and (*iii*) altered hydrology through dam construction.

We employed a sediment core top and bottom comparison approach, and can therefore only estimate water chemistry changes that have occurred between two discrete points in time: the present day and pre-1850. This top–bottom approach cannot infer trends that have occurred during the intervening period. Human activities may have caused more severe water-quality deterioration in the past. For example, the most severe perturbations in many of the lakes may have occurred during the initial land clearance activities of European immigrants (Fritz et al. 1993). Additional research is required to investigate trends through time and the impacts of these activities on lake water quality.

Conclusions

The relationships between surface-sediment diatom assemblages and lake water pH and [TP] were sufficiently strong to develop robust water-quality inference models based on weighted-averaging calibration and regression techniques. These diatom models were used to estimate water-quality changes, primarily acidification and lake trophic-status trends, that have occurred between the present day (1992) and preindustrial times in south-central Ontario lakes. Lakes that are presently acidic (i.e., pH ≤ 6.5) have generally acidified since the preindustrial period, most likely as a result of acidic atmospheric deposition. In fact, 33% of the presently acidic lakes had significant (>0.21 pH units) declines in diatom-inferred pH, whereas 12.5% had significant increases. In alkaline lakes (i.e., pH > 7), on the other hand, diatom-inferred pH has tended to increase during this period. Significant diatom-inferred pH increases were observed in 62.5% of the presently alkaline lakes, and none showed a significant increase. This alkalization has been observed in other regions affected by acid deposition (Sudbury, Ontario, and northeastern U.S.A.) and may result from watershed and in-lake processes that generate ANC. Circumneutral lakes showed no significant diatom-inferred pH changes.

In general, diatoms inferred relatively small changes in lake water [TP] in most of the study lakes since preindustrial times, despite modest shoreline development (mostly cottages) and past logging activity. Significant increases in diatom-inferred [TP] were observed in only 2 of the 54 lakes (4.5%), and they are currently oligotrophic. Eleven percent of presently oligotrophic lakes showed a significant decline in diatom-inferred [TP]. In half of the mesotrophic lakes (present-day [TP] > 10 $\mu g \cdot L^{-1}$), diatoms inferred that preindustrial [TP] was significantly higher than at present. Several factors could possibly account for declines in lake water [TP] during the past 150 years, including (*i*) lake and (or) watershed acidification processes that reduce P loading, or that increase the rate of P loss from lakes, and (*ii*) a reduction in nutrient loading from the watershed owing to logging, because early successional forests (50–100 years following forest clearance) may retain nutrients more strongly than mature, old-growth forests.

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Seasonal patterns in the mortality of *Daphnia* species in a shallow lake

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Abstract: To assess the impact of predation by young-of-the-year (0+) fish on the population dynamics of *Daphnia* species, we made independent estimations of the mortality of *Daphnia* species during the year, and of the predation pressure exerted by the juvenile fish. Mortality of daphnids was computed using a model that allowed us to differentiate between different size-classes, while total fish consumption was estimated from the temperature-dependent daily weight increase and the population development of the 0+ fish. The predation pressure on the different size-classes of *Daphnia* species was estimated by combining the total fish consumption with estimates of the selective feeding behaviour of the fish. To make the estimates of fish consumption independent of our current (1989–1991) zooplankton data set, we estimated fish species-specific and fish length-dependent selectivity indices on zooplankton using a different data set (1976–1977). *Daphnia* population densities usually increased in spring and decreased rapidly in early summer. Predation by 0+ fish was not severe enough to explain the large mortality that caused the summer decline; later in the year most of the mortality in the larger size-classes of the daphnids (>1.0 mm) could be explained by fish predation.

Resumé : Pour mesurer l'impact de la prédation par les jeunes poissons de l'année (0+) sur la dynamique des populations des espèces de *Daphnia*, nous avons fait des estimations indépendantes de la mortalité des espèces de *Daphnia* pendant l'année, et de la pression de prédation exercée par les jeunes poissons. Nous avons calculé la mortalité des daphnies à l'aide d'un modèle qui nous permettait de différencier les classes de taille, tandis que la consommation totale des poissons était estimée à partir de l'augmentation de poids quotidienne dépendant de la température et du développement de la population de poissons 0+. Nous avons estimé la pression de prédation sur les différentes classes de taille de *Daphnia* en combinant la consommation totale des poissons à des estimations du comportement d'alimentation sélective des poissons. Pour rendre les estimations de la consommation des poissons indépendantes de notre série présente (1989–1991) de données sur le zooplancton, nous avons calculé les indices de sélectivité à l'égard du zooplancton qui sont propres à l'espèce de poisson et dépendants de la longueur des poissons, en nous servant d'une série différente de données (1976–1977). Les densités de la population de *Daphnia* augmentaient généralement au printemps et baissaient rapidement au début de l'été. La prédation par les poissons 0+ n'était pas assez forte pour expliquer l'importante mortalité qui causait le déclin estival; pendant le reste de l'année, la majeure partie de la mortalité chez les plus grandes classes de taille des daphnies (>1,0 mm) pouvait s'expliquer par la prédation des poissons.
[Traduit par la Rédaction]

Introduction

Population densities of many zooplankton species usually show distinct seasonal patterns in temperate lakes. This seasonal behaviour has been well studied, particularly for daphnids (e.g., Luecke et al. 1990). Typically, the densities of *Daphnia* populations peak in late spring and then decline in early summer. This decline is often associated in time with a distinct clear-water phase, with high water transparency, and with a low phytoplankton abundance. The clear-water phase is caused by the increased grazing pressure of the zooplankton as a result of the increase in densities (Lampert et al. 1986). This leads to a reduction in the phytoplankton densities, and often to a shift in the phytoplankton composition from small single-celled diatom species to larger cyanobacteria, resulting in deteriorating food conditions for the daphnids and consequently declining animal densities.

Not all lakes show a clear-water phase. Especially in eutrophic lakes no apparent changes occur in the phytoplankton composition, yet population numbers of *Daphnia* species still decline sharply in summer (Vijverberg and Richter 1982; Gulati 1990). Consequently, as food limitation is less obvious in eutrophic lakes an alternative hypothesis was formulated to explain the decrease in daphnid densities in the summer (Mills and Forney 1983; Hairston 1987): the temperature increase in spring leads to an increase in consumption of zooplankton by planktivorous fish. The rise in temperature also triggers the

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spawning of fish (Mooij et al. 1994), followed by the recruitment of large numbers of fast-growing juvenile fish. Consequently, there is a sharp increase in the predation pressure on daphnids, which causes the population densities of these zooplankters to decrease.

In Tjeukemeer, a highly eutrophic lake, young-of-the-year (0+) fish constitute approximately 80% of the total fish production and are the main vertebrate zooplanktivores (Vijverberg et al. 1990). Hence, when investigating the effect of fish predation on the population dynamics of cladoceran zooplankton it suffices to evaluate the effects of 0+ fish, especially as older year-classes of most fish species often shift to other, more benthic, prey (Lammens et al. 1985).

We investigated the validity of the predation-induced decline hypothesis by estimating the impact of predation by juvenile fish on the population dynamics of daphnids. We compared two almost independent estimates of the mortality of daphnids. First, we estimated the total mortality of the daphnids using a method similar to the one derived by Paloheimo (1974). Secondly, we estimated the consumption of daphnids by 0+ fish, combining data on individual fish growth, selectivity, and fish densities. The comparison of these two estimates yielded information on the impact of juvenile fish on the population dynamics of *Daphnia* species.

Studies such as this one, integrating data on zooplankton production and fish consumption, are relatively rare (e.g., Luecke et al. 1990; Rudstam et al. 1992; Mehner et al. 1995), and might be subjected to criticism from two sides. On the one hand it might be argued that the assumptions and simplifications necessary for the computations completely underrepresent real systems, and combinations of different methods and sampling strategies will yield nontestable or irreproducible results, whereas on the other hand other workers might find the methods used in these computational exercises too elaborate. However, as the methods used in this study are a formalization of what is often done by rules of thumb we feel that an approach such as the one presented here is a useful tool to obtain more knowledge of aquatic systems as a whole, and results of studies like this one could direct further research.

Materials and methods

Study area

Tjeukemeer is a shallow (mean depth 1.5 m), wind-exposed, eutrophic lake, with a surface area of 21.5 km^2 and a poorly developed littoral zone. Phytoplankton biomass in the lake is high during summer, with chlorophyll *a* concentrations often exceeding 100 $\mu g \cdot L^{-1}$. Algal biomass is dominated by diatoms in February and March and by cyanobacteria, mainly *Oscillatoria* species, during the rest of the year. In general, no clear-water phase occurs; summer Secchi-disc depths vary between 25 and 35 cm.

The herbivorous zooplankton is dominated by cladocerans: *Bosmina coregoni, Bosmina longirostris, Chydorus sphaericus, Ceriodaphnia pulchella, Daphnia galeata, Daphnia cucullata*, and the hybrid between these two daphnid species. Invertebrate predators include *Leptodora kindtii*, different species of omnivorous cyclopoid copepods (*Cyclops vicinus, Acanthocyclops robustus*, and *Mesocyclops leuckarti*), and water mites. The opossum shrimp, *Neomysis integer*, is usually present in low densities (Bremer and Vijverberg 1982), while larvae of the phantom midge, *Chaoborus* sp., are almost absent from Tjeukemeer.

The open-water fish community of the lake consists of eight species: bream (*Abramis brama*), white bream (*Blicca björkna*), roach (*Rutilus rutilus*), smelt (*Osmerus eperlanus*), pikeperch (*Stizostedion lucioperca*), perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), and eel (*Anguilla anguilla*). Although adult bream has by far the largest standing stock of the fish species in Tjeukemeer, production is lower than the production of the 0+ fish (Vijverberg et al. 1990). As a result, young-of-the-year smelt, bream, perch, pikeperch, and roach are the main zooplanktivores in Tjeukemeer. In most years smelt constitute 70–80% of the 0+ fish biomass.

Sampling

Zooplankton

During the years 1976–1977 and 1989–1991 zooplankton was sampled with a 5-L Friedinger sampler at five stations. At each station two samples were taken, one below the surface and the other just above the bottom of the lake. Sampling was done at weekly intervals during the growing season from April to October, and at fortnightly intervals during the rest of the year. The samples were concentrated by filtration through a 120-μm mesh sieve, pooled, and preserved in a 4% formaldehyde solution. The cladoceran zooplankters were identified to species, whereas the copepods were classified as cyclopoids and calanoids, and the densities were established. For the numerically important groups, length–frequency distributions were determined by measuring 100 individuals. Daphnids were measured from the top of the eye to the base of the tail spine. For the other cladocerans the total length minus the tail spine was measured. Because copepods usually have curved telsons in fish stomachs, or break in two, cephalothorax lengths were recorded for these groups. To compute birth and death rates the relationships between egg number and animal length were established for the *Daphnia* species on animals taken in additional samples that were stored in 95% ethanol.

Fish

Length–frequency distributions of the five major 0+ fish species were obtained from a routine sampling of fish in Tjeukemeer, which started in 1975 (Lammens et al. 1990). Trawling was done monthly at the same five stations in the open-water zone of the lake as the zooplankton samples were taken, with a 5-m small-mesh beam trawl (5-mm cod end, fishing speed 1 $m \cdot s^{-1}$, 10-min hauls). The 0+ fish were sorted, identified, and measured to the nearest 0.5 cm. Average numbers caught per sampling date were calculated using the geometric mean of the five stations (Buijse 1992). To estimate the absolute numbers of fish, the trawl catches were calibrated in 1976 using a purse seine (Coles et al. 1985) with an encircled area of 76.5 m^2, assuming that with this type of netting 100% of the juvenile fish were caught. The calibrations were made in the growing season of the juvenile fish. No indications were found that the efficiency of the trawl net for juvenile fish changed during the course of the season.

In 1976, 0+ fish were caught at fortnightly intervals during the growing season and measured. The stomach contents of individual fish were analyzed, and the species and size

distributions of the prey in the stomach were established (van Densen 1985).

Daphnia mortality

The mortality of the different *Daphnia* species was estimated using the discrete event model INSTAR (Hogeweg and Richter 1982; Vijverberg and Richter 1982; Hovenkamp 1990). The model simulates a population of zooplankton, using field data on fecundity and length–frequency distributions, and estimates the number of individuals of different size-classes that die in a certain period by comparing the computed numbers with the numbers observed in the field. If the numbers in the model are higher than the observed densities in the field, this difference in numbers is removed from the model population and these animals are assumed to have died. If the numbers in the model are lower, mortality is set to zero; no hatching from resting stages is included in the model. Modelled numbers are, however, only rarely lower than those in the field. Field data were smoothed prior to the application of INSTAR by applying a three-point moving average.

The model is essentially the same as the model to compute death rates described by Paloheimo (1974), which uses the equation $r = b - d$, in which r is the natural rate of increase, b is the birth rate, derived from the egg development time and the average number of eggs in the population, and d is the death rate. The difference is that with the help of INSTAR different size-classes can be considered, which can result in different death rates for these different size-classes. The somatic growth rates determine the transition speed from one size-class to the other, and hence the outcome of the analyses depends on the estimated growth rates. As a result of the continuous recruitment of the animals, it is not possible to measure growth of daphnids in the field. It is, however, possible to establish the size at maturity under field conditions. The size of the smallest individuals with eggs was taken as an estimate of the size at maturity in the field. By using laboratory-derived data on the relative growth of the juvenile instars (Boersma and Vijverberg 1994*a*) we computed the growth of the different juvenile instars. The ratio between the size at maturity and the maximal size an animal can reach was estimated using laboratory observations (Vijverberg 1976; Lei and Armitage 1980; Geller 1987; Urabe 1988; Boersma and Vijverberg 1994*a*) to be a value averaging 0.62. Assuming that the size at maturity is influenced only by the ambient conditions, we were able to estimate the maximum length of the animals in the field. Using a von Bertalanffy growth equation, the growth of the animals in the field was established. Hence, depending on the size at maturity of the animals in the field, different growth rates were used in the model, with small size at maturity reducing the somatic growth (see also Taylor and Slatkin 1981).

Mortality rates were estimated separately for *D. galeata*, *D. yugcucullata*, and their hybrid. To compare the computed densities with the field densities, eight size-classes were arbitrarily chosen, with a size-class interval of 0.125 mm. In the generated output, length classes were merged to obtain four output classes: (*i*) <0.5 mm, (*ii*) 0.5–1.0 mm, (*iii*) 1.0–1.5 mm, and (*iv*) >1.5 mm. Because zooplankton was sampled every week, computing mortality on a daily basis suggests an accuracy that cannot be sustained by the original data. However, the period between the two dates at which mortality is computed should not be too long either, as this would cause an overestimation of the mortality rates of the larger size-classes. Therefore, mortality was computed twice a week by comparing the computed densities with interpolated field data, and reports were generated on a weekly basis.

Fish consumption

The consumption of different zooplankton species by juvenile fish was estimated using data on prey selectivity, fish growth, and densities of the different fish species.

Selectivity

The preferences of the 0+ fish for different zooplankton species and size-classes were estimated using the data set collected by van Densen (1985) in the years 1976–1977, consisting of detailed analyses of the gut contents of 0+ fish in relation to their own length and to the zooplankton densities. Food preferences were established by computing the selectivity index (α) of Chesson (Chesson 1978, 1983) for every individual fish and every prey class. Chesson's α is defined as the proportion of prey class i in the gut, r_i, divided by the proportion of prey class i in the environment, p_i, normalized in such a way that the sum of the α values over all prey types equals 1, or

$$\alpha_i = \frac{(r_i/p_i)}{\sum(r_j/p_j)}$$

Smelt, perch, and pikeperch hardly forage at all on the smaller zooplankton species, such as *Ceriodaphnia pulchella*, *Bosmina coregoni*, *Bosmina longirostris*, and *Chydorus sphaericus* (van Densen 1985). If the smaller species are eaten at all, the numerical proportion of these species in the gut is usually low. This, combined with their small size and hence low individual biomass, results in a small proportion of the fish growth being attributable to the small cladocerans. Therefore, we omitted the smaller cladocerans from the analysis of the prey selectivity of smelt, pikeperch, and perch, and for these species we distinguished seven prey classes: copepods in two length classes (<0.5 mm, >0.5 mm), daphnids in four different length classes (<0.5 mm, 0.5–1.0 mm, 1.0–1.5 mm, >1.5 mm), and *L. kindtii* in one length class, as the densities of this latter species were too low to establish a length–frequency distribution.

0+ bream prefer smaller prey items (van Densen 1985), and hence the smaller cladocerans were incorporated in the analysis of the gut contents of 0+ bream. As data on stomach contents of 0+ bream were scarce, we were not able to discriminate between size-classes for the estimations of selectivity. Hence, only species-specific prey classes were used: copepods, daphnids, *Bosmina coregoni*, *Bosmina longirostris*, *Ceriodaphnia pulchella*, and *Chydorus sphaericus*. Stomach content data for roach were not available in adequate numbers to warrant proper analysis, and thus this species was omitted from the analyses.

The dependence of α on fish length was estimated for the different fish species, using a logit regression technique (McCullagh and Nelder 1983). The length and square length of the fish were used as independent variables and Chesson's α was used as the dependent variable. Thus,

$$\text{logit}(\alpha_i) = \log\left(\frac{\alpha_i}{1-\alpha_i}\right) = k + l \cdot L + m \cdot L^2 + \varepsilon$$

in which k, l, and m are the parameters to be estimated, L is the length of the fish, and ε is the error term. To obtain the best fit through the data we maximized the probability of finding the actually observed numbers of the different prey items in the guts given the estimated values of α. This was accomplished by minimizing the −2log(likelihood) values of the observed numerical composition of the gut content in an iterative process, in two steps. First, the dependence of α on fish length was estimated for each prey class separately. Subsequently, a multinomial fit was obtained by analyzing all prey classes per fish species together, constraining the sum of the Chesson's α values at any fish length to be close (the difference being smaller than 0.001 for smelt and smaller than 0.0005 for the other species) to the expected value of the sum of α over all prey classes, which is 1. To avoid weighting large fish (with many prey items in the gut) too heavily in the analysis compared with the smaller fish (with only a few prey items), we chose to weight the gut data with the reciprocal value of the total number of prey items in the gut, i.e., every individual fish had the same weight in the analysis. Constraining the sum of the α values means that it is difficult to estimate the accuracy of the parameters k, l, and m. Therefore, only the significance of the total regression model was computed by comparing the −2log(likelihood) estimates of the full model with the −2log(likelihood) estimates of the simpler models, that is, with the model with r_i constant, meaning that the proportion of all prey types in the gut is independent of the length of the fish and of the proportions of the prey types in the environment. Furthermore, the total regression model was compared with the model with α_i constant, implying that the preference for certain prey types would not change during the ontogeny of the fish. As differences in −2log(likelihood) values are χ^2 distributed, the significance of the different models in relation to each other can be tested.

The α functions derived from van Densen's (1985) data set were used to estimate the consumption of zooplankton by juvenile fish in the years 1989–1991.

Fish growth

As we observed a significant effect of the length of the fish on their preferences for certain food types, estimates of the length of the different fish species were necessary for the estimation of the consumption rates in 1989–1991. Fish lengths were obtained by fitting the temperature-dependent growth model of Mooij and van Tongeren (1990) through the observed lengths of the juvenile fish as caught in the monthly field samples. The model of Mooij and van Tongeren has been shown to predict the field observations of 0+ fish length accurately (Mooij et al. 1994). Growth was computed as the resultant of feeding conditions, body weight (W), and water temperature (T):

$$\frac{dW}{dt} = a \cdot W^b \cdot (T - c)$$

The model has three parameters: a food (a), weight (b), and temperature (c) parameter. It is not to be expected that the values of b and c will vary between years, as these parameters are intrinsic properties of the fish species. Hence, we used the b and c values for 0+ fish growth in Tjeukemeer in the years 1976–1988, which were estimated by Mooij et al. (1994) (Table 4), with $b = 0.6$. The food parameter, a, was estimated for each species for each year, to obtain an optimal fit of the growth model through the field data. We found that in 1989 the temperature- and weight-corrected growth rates of all species decreased sharply after 15 July. Therefore, we calculated two values for a for this year, one for the feeding conditions before, and the other after, 15 July.

Fish densities

The changes in densities of 0+ smelt, pikeperch, and perch in the pelagic zone of Tjeukemeer were described using the familiar negative exponential equation:

$$N_t = N_0 \cdot e^{-zt}$$

The numbers per sampling date of the juvenile cyprinids, that is, bream and roach, usually increase during July and August, which can be explained by a migration of these species from the littoral zone to the pelagic zone in this period (Mooij 1992). The best fit through the data of the cyprinid abundances was given by the following equation:

$$N_t = N_0 \cdot e^{-(z + yt)t}$$

The data on fish abundance were too sparse to estimate the mortality parameters z and y for each year separately. Therefore, 3-year averages of z and y were computed for every fish species. With the use of the parameters z and y, a standardized abundance for each species at 1 September in every separate year (N_0) was calculated.

Consumption

The growth model of Mooij and van Tongeren (1990) essentially computes daily weight increments. Using a conversion efficiency of 0.12 (O.F.R. van Tongeren, unpublished results) between the growth of the fish and the zooplankton consumption needed for this growth, we computed the daily zooplankton consumption per fish.

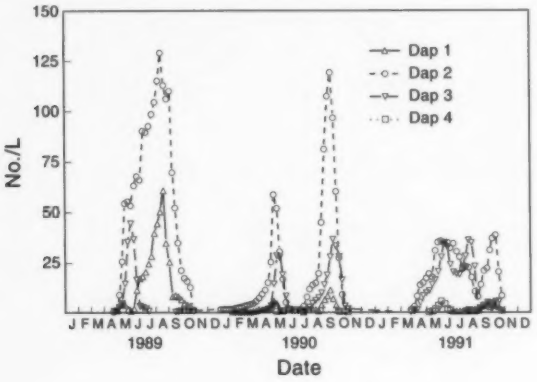
The combination of the computed lengths of the 0+ fish and the proportions of the different prey classes in the lake at the time with the relationships of α values with fish length as computed from the 1976 data set yielded the proportions of the different prey classes in the guts of individual fish in 1989–1991. These expected proportions in the fish gut were expressed as numerical proportions, whereas total food uptake was expressed in mass units. The average weight of the zooplankters in the gut was estimated using the average mass of the different prey classes, weighted by their numerical proportions in the gut. Dividing the mass of the food consumed by each individual fish by the average prey mass gave the total number of consumed prey, which was then divided over the different prey classes using the expected numerical proportions.

Combining the densities of the juvenile fish with the consumption per fish yielded an estimate of the total mortality of the zooplankton caused by juvenile fish.

Assumptions

Computational exercises, such as the one presented here, are based on several assumptions. In many cases, however, these assumptions are not mentioned, making results of the

Fig. 1. Seasonal variation in the densities of different size-classes of *Daphnia* species in 1989–1991 in Tjeukemeer (Dap 1, <0.5 mm; Dap 2, 0.5–1.0 mm; Dap 3, 1.0–1.5 mm; Dap 4, >1.5 mm).



computations difficult to interpret. To avoid such difficulties in interpretation we made the assumptions underlying our computations explicit.

(*i*) *Daphnia* species were lumped while considering predation by fish. Hence, we assume that 0+ fish feed aselectively on different species of *Daphnia* within a given size-class.

(*ii*) We were selective in the possible prey items for the 0+ fish species we included in our computations. For smelt, pikeperch, and perch the smaller zooplankton species were not considered as prey. Naturally this influenced the results, because by attributing all of the growth of the juvenile fish to daphnids, copepods, and *L. kindtii*, the predation pressure on the different size-classes of *Daphnia* species was most likely overestimated. However, as the biomass contribution in the fish gut of the smaller zooplankton species was small (<5%) (van Densen 1985), the size of this bias is likely to have been small.

(*iii*) No length measurements were available for the prey items of 0+ bream. Because of the high preference of 0+ bream for smaller (<1.0 mm) zooplankton species we assumed that the predation of 0+ bream on *Daphnia* species was limited to individuals of size-class 2 (0.5–1.0 mm).

(*iv*) By using the value of the selectivity indices of 1976–1977 for the years 1989–1991 we assumed that the fish length – α relationships did not change between these years. During these 13 years the lake hardly changed: fish, zooplankton, and phytoplankton communities were stable, no new fish or zooplankton species were introduced, and no species disappeared. Therefore, there is no reason to expect changes in fish feeding preferences.

(*v*) Prey densities were not used as independent variables in the computations; only the proportions of the different prey items in the field were incorporated as independent variables in our analyses. This translates to the assumption that optimal foraging does not play a role in this predator–prey system. Although evidence exists that optimal foraging in fish may be of importance (e.g., Mittelbach 1981; Persson and Greenberg 1990), the impact of optimal foraging strategies on the diets of fish is as yet not clear (e.g., Mills et al. 1986).

(*vi*) We assumed that the conversion factor between fish growth and food uptake was equal for the different fish species, similar for all different prey types, and constant over the temperature range found in the lake in summer. A conversion efficiency of 0.12 between food uptake and growth of the fish was used (O.F.R. van Tongeren, unpublished results). The conversion efficiency may seem low when compared with the published values for well-fed carnivorous juvenile fish of 0.2–0.3 (Brett and Groves 1979). However, older planktivorous fish have a lower conversion efficiency (0.12; O'Grady and Spillet 1985). Moreover, Mills et al. (1989) reported conversion efficiencies for 0+ perch that were even lower (<0.10). All of the juvenile fish in this study are planktivorous, and we therefore feel that this low conversion efficiency is justified. By keeping the conversion factor at a fixed level over the temperature range some bias was probably introduced, because the conversion efficiency is known to decrease with increasing temperature. However, during the growing season of juvenile fish in Tjeukemeer the temperature of the lake is relatively constant (14–19°C). Moreover, as little is known about the exact relationships of the conversion efficiency of these different fish species with temperature, we chose to keep the values for the conversion efficiency constant. Had we included a change in conversion efficiencies with temperature, then the predicted predation rates in spring would have decreased, and the predicted predation rates in the summer months would have increased.

(*vii*) The data on fish abundance were too sparse to estimate the mortality parameters z and y for each year separately. Given the observation that the mortality of 0+ fish is mainly caused by predation (Mooij 1992), and that the densities of the main piscivorous fish, pikeperch, were similar in the years 1989–1991, we presumed that the shapes of the mortality curves were similar between the years, and hence 3-year averages of z and y were computed, meaning that the years 1989–1991 only differed in initial fish densities.

(*vii*) To be able to make the comparison between the absolute estimates of predation by fish and of mortality we had to compute the absolute densities of juvenile fish in the lake. This was done using trawl data and the net efficiency established for juvenile fish during the course of the year. As this calibration set was only small, it could be argued that using one net efficiency for all fish species, length classes, dates, and weather types is not appropriate. However, as we did not find a change in net efficiency, and temporal reproducibility of yields in trawl catches is high (Mooij 1992), differences in net efficiency are not likely to have been large.

Results

Daphnia

Daphnia size-class 2 (0.5–1.0 mm) dominated the size spectrum in 1989 and 1990, whereas size-class 3 (1.0–1.5 mm) contributed substantially to the total densities in 1991 (Fig. 1). These interannual differences were mainly the result of the varying densities of the different *Daphnia* species. In 1989 we observed a succession of *D. galeata* by the smaller hybrid, *D. galeata* × *cucullata*, which was again succeeded by the still smaller *D. cucullata*, responsible for the peak in densities of the smallest size-class in the summer of 1989. In 1990 the

Fig. 2. Mortality of the different size-classes of *Daphnia* species in Tjeukemeer, expressed as the number of daphnids that died per litre per week.

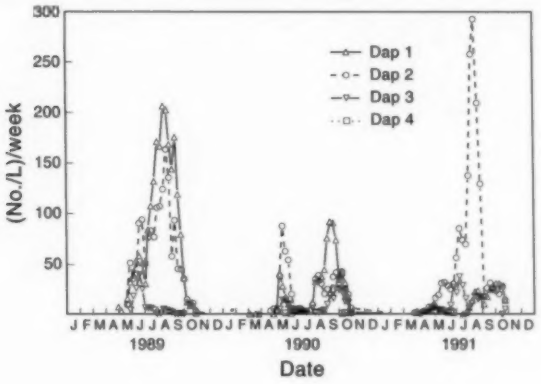


Table 1. Correlation coefficients (r) and their significance (P) between the per capita rates (d^{-1}) of the different size-classes of the daphnids.

Size-class	Dap 1		Dap 2		Dap 3	
	r	*P*	*r*	*P*	*r*	*P*
Dap 2	0.37	0.001				
Dap 3	−0.13	0.195	−0.08	0.410		
Dap 4	−0.01	0.992	−0.08	0.527	0.27	0.030

Note: For definitions of the size-classes see the caption of Fig. 1.

Table 2. Correlation coefficients (r) between the per capita death rates (d^{-1}) of the different size-classes of the *Daphnia* species with the rate of increase of the populations at the time.

Size-class	*D. galeata*			*D. galeata* × *cucullata*			*D. cucullata*		
	r	*P*	*N*	*r*	*P*	*N*	*r*	*P*	*N*
Dap 1	−0.17	0.542	15	−0.30	0.003	98	−0.10	0.387	85
Dap 2	−0.35	0.001	84	0.11	0.266	101	0.02	0.882	86
Dap 3	−0.14	0.188	84	0.09	0.938	84	0.12	0.418	48
Dap 4	−0.10	0.477	57	−0.41	0.011	38			

Note: For definitions of the size-classes see the caption of Fig. 1.

spring peak was dominated by *D. galeata* and the hybrid, whereas the autumn peak mainly consisted of *D. cucullata* and the hybrid. *Daphnia galeata* dominated the whole year of 1991, except for the small peak of size-class 2 late in the year, which was mainly the hybrid (see also Spaak 1994).

Mortality, expressed as the number of animals that died per litre in each week, differed greatly between the size-classes, and was not correlated to the densities in the field (Fig. 2). Animals in the smallest size-class showed a high mortality, whereas mortality in the largest size-class was much lower. A substantial difference in mortality patterns was found between years. To make the mortality figures between size-classes more comparable, per capita death rates were computed by dividing the mortality by the densities of the different size-classes. Per capita death rates (d^{-1}) for the smallest two size-classes were positively correlated, as were the rates for the largest two size-classes. The other correlations were not significantly different from zero, although all were negative (Table 1). Both *D. galeata* and *D. galeata* × *cucullata* showed significantly negative correlations between their respective population growth rates, r, and the per capita death rates in their smallest size-class (Table 2). Only a few of the juvenile *D. galeata* were smaller than 0.5 mm, so for this species the smallest size-class is class 2. No significant correlation was found between any of the per capita death rates of *D. cucullata* and the rate of population increase in this species. Only for *D. galeata* × *cucullata* was the per capita death rate of the largest size-class negatively correlated with r.

Table 3. Year-specific log-transformed densities per litre on 1 September ($\ln(N_0)$) and average rates of change in the population (z (d^{-1}) and y (d^{-2}) in the equation $N_t = N_0 \cdot e^{-(z+yt)t}$) of the five major 0+ fish species in Tjeukemeer during 1989–1991.

	Smelt	Pikeperch	Perch	Bream	Roach
$\ln(N_0)$					
1989	−7.71	−10.55	−7.07	−6.94	−9.15
1990	−7.59	−12.76	−13.45	−8.81	−10.51
1991	−6.16	−12.15	−12.13	−7.61	−10.29
z	0.0157	0.0374	0.0060	−0.017 7	0.001 8
y	0	0	0	0.000 80	0.000 76

Table 4. Year-specific food parameters, a ($\times 10^{-3}$ $g^{0.4} \cdot °C^{-1} \cdot d^{-1}$), of the five major 0+ fish species in Tjeukemeer during 1989–1991.

	Smelt	Pikeperch	Perch	Bream	Roach
a_{89a}	1.34	5.26	6.15	8.84	5.88
a_{89b}	1.11	2.17	1.86	6.31	3.35
a_{90}	1.26	3.25	4.68	6.45	3.99
a_{91}	1.13	4.20	4.55	6.21	3.84
a_{76-88}	1.10	4.07	4.40	5.62	3.60
c	−3.6	9.8	9.8	12.8	10.2

Note: For 1989, two food parameters were estimated (a_{89a} and a_{89b}), representing the feeding conditions before and after 15 July, respectively. Long-term values of a (a_{76-88}, $\times 10^{-3}$ $g^{0.4} \cdot °C^{-1} \cdot d^{-1}$) and of the temperature parameter, c (°C), are also presented. Parameter b was kept constant at 0.6 for all species and all years (Mooij et al. 1994).

0+ fish

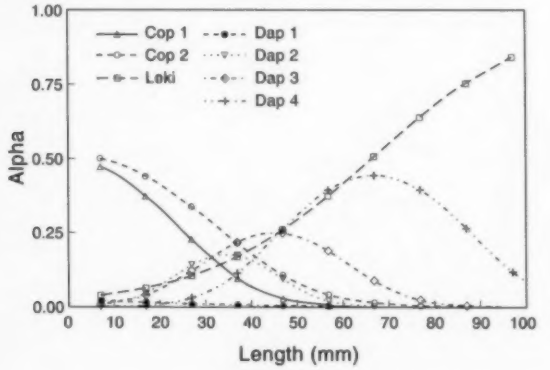
The total abundance of 0+ fish varied annually, and was four times higher in 1989 than in 1990 (Table 3). Fish densities in 1991 were intermediate between those in 1989 and 1990. In 1989 the 0+ fish community was dominated by smelt, perch, and bream in fairly equal numbers. In 1990 and 1991 smelt dominated the 0+ fish community; pikeperch and perch were almost absent in these years. Roach was present in low numbers in all 3 years, whereas the other fish species showed large interannual differences. It can be seen from Table 3 that the mortality of pikeperch was the highest of the species under consideration in 1989–1991, as the z value for pikeperch was

Table 5. Parameters of the length-logit transformed α regressions for the different fish species.

Prey class	Parameter	Smelt	Pikeperch	Perch
Copepods				
<0.5 mm (Cop 1)	*k*	0.003 7	1.275 0	0.085 1
	l	–0.005 1	–0.168 2	–0.051 0
	m	–0.001 5	–0.001 0	–0.000 1
>0.5 mm (Cop 2)	*k*	0.054 9	–2.350 2	–1.189 1
	l	–0.000 95	0.137 8	0.010 1
	m	–0.000 97	–0.003 1	–0.000 01
Daphnids				
<0.5 mm (Dap 1)	*k*	–3.674 4	–1.729 4	–2.714 5
	l	–0.020 9	–0.189 7	–0.032 2
	m	–0.000 48	–0.001 0	–0.000 7
0.5–1.0 mm (Dap 2)	*k*	–7.184 1	–3.769 0	–2.669 4
	l	0.326 2	0.071 1	0.055 1
	m	–0.004 7	–0.001 2	–0.000 1
1.0–1.5 mm (Dap 3)	*k*	–6.458 6	–3.739 9	–2.409 0
	l	0.236 6	0.082 4	0.065 1
	m	–0.002 6	–0.001 4	–0.000 6
>1.5 mm (Dap 4)	*k*	–9.343 1	–3.955 0	–3.641 1
	l	0.271 4	0.088 7	0.043 4
	m	–0.002 0	–0.000 8	–0.000 01
Leptodora kindtii				
(Leki)	*k*	–3.043 9	–1.371 1	–3.255 8
	l	0.043 0	0.043 7	0.014 2
	m	0	0	0

Note: The parameters indicate the constant (*k*), and the regression coefficients for length (*l* (mm^{-1})) and square length (*m* (mm^{-2})).

Fig. 3. Feeding selectivity of smelt (*Osmerus eperlanus*) in relation to its own length (Cop 1, copepods <0.5 mm; Cop 2, copepods >0.5 mm; Dap 1, daphnids <0.5 mm; Dap 2, daphnids 0.5–1.0 mm; Dap 3, daphnids 1.0–1.5 mm; Dap 4, daphnids >1.5 mm; Leki, *Leptodora kindtii*.



the largest. The year-specific parameters of the fish-growth model are summarized in Table 4.

Zooplankton and 0+ fish

The parameters of the length-logit transformed α regressions are given in Table 5 for smelt, pikeperch, and perch. Small fish

Table 6. Parameters of the length-logit transformed α regressions for bream for different prey species.

Prey class	Parameter	Bream
Copepods	*k*	0.858 9
	l	–0.161 3
	m	0.001 0
Daphnids	*k*	–1.572 7
	l	0.017 2
	m	–0.000 01
B. coregoni	*k*	–1.887 3
	l	0.037 3
	m	–0.000 3
B. longirostris	*k*	–3.412 4
	l	0.015 3
	m	–0.000 01
C. sphaericus	*k*	– 1.643 4
	l	–0.003 2
	m	–0.000 1
C. pulchella	*k*	–0.871 3
	l	0.014 3
	m	–0.000 7

Note: The parameters indicate the constant (*k*), and the regression coefficients for length (*l* (mm^{-1})) and square length (*m* (mm^{-2})).

Table 7. Number of fish (*N*) used for the gut analysis and –2log(likelihood) values of the models: r_i constant, α_i constant, and α_i dependent on the length of the fish.

	Smelt	Pikeperch	Perch	Bream
N	148	167	88	14
r_i constant	2026	2286	1342	44
α_i constant	523	528	305	48
α_i length dependent	408	510	291	44

Note: The number of degrees of freedom in the comparisons of the different models, r_i constant versus α_i constant, and α_i constant versus α_i length dependent, were 7 and 13, respectively.

of all three species showed the highest α values for the two copepod classes (Fig. 3). With an increase in length, α values for increasing size-classes of daphnids showed an optimal curve, although α for the smallest daphnids was low over the whole length range. At around 65 mm in length, smelt started to prefer *L. kindtii* as prey (Fig. 3). The preference for the largest prey class began at smaller lengths for pikeperch. Perch showed high and constant α values for the copepod 2 class. Juvenile bream showed a high preference for the smaller cladocerans (Table 6). In the case of smelt, pikeperch, and perch the –2log(likelihood) values of the length-dependent α regressions were significantly lower than the –2log(likelihood) values of the constant r_i regressions (Table 7). In addition, the –2log(likelihood) values of the length-dependent α regressions were also lower than the –2log(likelihood) values of the constant α_i regressions. The difference, however, was only significant for smelt. It should be kept in mind, however, that the values for χ^2 (being the differences between the

–2log(likelihood) values for the different models) are a severe underestimation, as a result of the weighting of the data, implicitly assuming that all fish only consumed one prey item. With two prey items consumed per fish the χ^2 values would already be doubled, yielding significant differences in all cases.

When the numbers of *Daphnia* spp. eaten by juvenile fish were compared with the total numbers of *Daphnia* spp. that died in the same period the different size-classes showed distinctly different patterns (Fig. 4). Animals in the smallest size-class (<0.5 mm) were not eaten by juvenile fish, yet they showed a high mortality. The mortality in the larger classes can be explained better by predation by fish; in the second half of 1990 and 1991 all of the mortality in the larger size-classes of the daphnids can be explained by predation by 0+ fish. If a peak in mortality in the larger classes occurs early in the year, as in 1990, juvenile fish predation cannot explain the mortality at that time.

Although the number of daphnids eaten by fish in the larger size-classes was close to the number that died in the second part of the year, some differences were found mainly in the period from September to October in the 3 years. Age 0+ fish showed marked increases in preference for *L. kindtii* with length (Fig. 3), having the greatest preferences at the end of the year. However, because of the low densities of *L. kindtii* in the lake, numbers caught in the standard zooplankton sampling programme were small, and hence the determination of densities was inaccurate. Because of the high α values for this prey item, the proportion of *L. kindtii* in the environment greatly influences the predicted predation rates on all alternative prey classes, and hence it is important to have a good estimate of its density. To assess the impact of inaccuracies on the estimates of *L. kindtii* densities, we reanalyzed the data with the observed densities of *L. kindtii* both plus and minus two standard deviations. Expected numbers in a sample are Poisson distributed, and hence the square root of the number of individuals counted is an estimate of the standard deviation. From the analysis it became clear that the density of *L. kindtii* as an alternative prey played an important role in the predicted predation rate on the larger size-classes of daphnids later in the year.

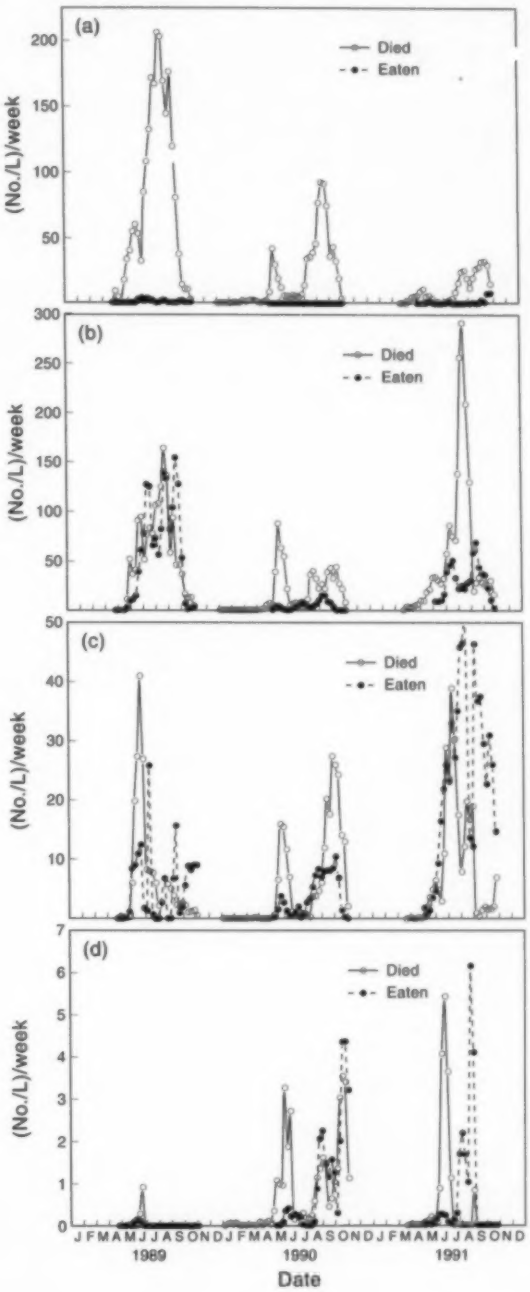
Discussion

Approach

The two ways of estimating mortality were almost completely independent. Consumption by fish was estimated using fish growth and fish densities obtained from the field, combined with a measure of prey selectivity, which was estimated using observations from 1976–1977, years with different fish densities and zooplankton numbers. *Daphnia* mortality was estimated using only the *Daphnia* densities and fecundities, in combination with some laboratory-derived parameters of the growth and temperature dependence of the growth and reproductive processes. The only data used for both the mortality and the consumption estimates were the densities of the different *Daphnia* size-classes. It is important to note, however, that in the estimates of consumption the densities were not used as such, but as proportions of the different prey classes in the environment, although these two were positively correlated (r^2 values between densities and proportions of the different prey groups varied between 0.04 and 0.72).

Given the assumptions underlying the computations of the

Fig. 4. Total mortality of daphnids (number of individuals per litre per week) in size-classes 1 (*a*), 2 (*b*), 3 (*c*), and 4 (*d*), related to the predation mortality of 0+ fish. For definitions of the size-classes see the caption of Fig. 1.



mortality of the daphnids on the one hand and the consumption by the juvenile fish on the other hand it is remarkable that the results obtained from the two different ways of estimating the mortalities are of the same order of magnitude. However, it would be unwise to elaborate on the absolute magnitude of the predation and mortality rates reported here. It is difficult to assess the reliability of estimates in studies such as this one, which is illustrated by the fact that in similar studies no (Luecke et al. 1990) or very limited (Rudstam et al. 1992) attempts were made to estimate the reliability of the results. Sampling variation in the density determination of the different species is most probably driving the accuracy of the model output, with coefficients of variation estimated for zooplankton of approximately 15% (de Nie and Vijverberg 1985), a value lower than the ones reported for a large number of lakes by Pace et al. (1991). Sampling variation in the fish densities was higher, with a coefficient of variation of 60%. We have, however, most likely overestimated the predation by juvenile fish, as a result of the low conversion efficiency between food uptake and growth, as a result of the exclusion of some prey types, and as a result of the fact that the conversion efficiency was estimated in the laboratory at 20°C (O.F.R. van Tongeren, unpublished results). This means that in all periods in the 3 years with lower temperatures, predation will have been overestimated. Nevertheless, some general conclusions can be drawn from our results as these reflect differences in mortality rates and consumption rates of an order of magnitude.

Predation of 0+ fish

Given the limitations of the computations presented here, two main conclusions can be drawn from our results. Firstly, the smallest size-class of *Daphnia* (<0.5 mm) had a high mortality, but the consumption by 0+ fish of this size-class was almost zero. Thus, the cause of the mortality of this size-class cannot be explained by fish predation. Small *Daphnia* individuals have the lowest starvation resistance (Threlkeld 1976), and hence starvation could be responsible for the high juvenile mortality, especially because daphnids are food limited during most of the year (Boersma and Vijverberg 1994*b*). Moreover, smaller daphnids are more vulnerable to invertebrate predation than larger ones (e.g., Mordukhai-Boltovskaia 1958; Herzig and Auer 1990). Relatively little is known, however, about the selectivity and consumption rates of the invertebrate predators present in Tjeukemeer: *L. kindtii*, cyclopoid copepods, and water mites. Although Hovenkamp (1990) found that the mortality in the smallest size-classes of *Daphnia* species could be attributed to predation by *Chaoborus flavicans* and *L. kindtii* in mesotrophic Lake Vechten, and others have also reported large impacts of predation by invertebrates on the community structure of the herbivorous zooplankton (e.g., Kerfoot 1977), it is unlikely that the predation rates of the invertebrate predators in Tjeukemeer were high enough to explain the high mortality rates of the small daphnids. If we assume that each *L. kindtii* individual consumes 10 prey individuals per day (see Browman et al. 1989), and it does this aselectively, then it can be computed that predation by *L. kindtii* is too low to explain the high mortalities of daphnids (as was also concluded by Lunte and Luecke 1990): the densities of the smaller cladocerans are usually also high during the growing season of *L. kindtii*, which will most likely lead to a high predation pressure of *L. kindtii* on the smaller species. The same is true for the predation by copepods, whereas the effect of the predation by water mites cannot be inferred, as no information is available on this group of predators in Tjeukemeer.

Our own observations showed that in cultures intrinsic mortality was highest when the animals were small; moreover, small daphnids seemed to be most vulnerable to physical disturbances. Herzig (1974) reported that death rates of *Diaphanosoma brachyurum* were strongly positively correlated with wind speed, whereas McNaught and Hasler (1961) reported large amounts of air-locked *Daphnia* species in the foam lines of Langmuir circulations (see also George and Edwards 1973). Fryer (1991) also reported patches of millions of doomed *Daphnia* individuals trapped in the surface film of the water. The fact that we do find a significantly negative correlation between the mortality of the smallest size-classes and the population growth rates in two of the three taxa (Table 2) does show that the mortality rate in this size-class is important for the population dynamics of the daphnids.

The second conclusion that can be drawn from this study is that the larger size-classes of the daphnids were hardly eaten at all in the first part of the year, although the mortality rate accompanying the end of the spring peaks was high in 1990. The utilization of the daphnids by 0+ fish early in the year is dependent on the timing of the population growth of the daphnids. If the growth starts early in the year, when the juvenile fish are not yet present, the zooplankters will overexploit their resources, and the population densities will decrease as a result of food limitation. Hence, it is not possible to explain the collapse of the spring densities by predation by juvenile fish. Irrespective of whether the 0+ fish are present or not, *Daphnia* populations will decline. The same observation was made by Luecke et al. (1990), who also found high mortality in spring, when fish predation is too low to explain this mortality.

It is important to note that even if all of the zooplankton mortality could be explained by fish predation, it would be incorrect to conclude that the population dynamics of the particular species under consideration is the only factor of importance. After all, in the computation of the population development of the zooplankton, field-derived length–fecundity relationships were used. These will greatly affect the computed birth rates, and hence the computed densities, implying that comparisons of mortality data for zooplankton will always underestimate the importance of the food conditions. In our case, the sharp decrease in fecundity at the time of the summer decline remained invisible; only the increased mortality at this time became apparent. Usually, the declines in birth rates are used to explain the decline in numbers in spring (e.g., Sommer et al. 1986). However, as we have shown here, even with the decrease in birth rates accounted for, mortality was also high at the time of the decline. This suggests that in this period the daphnids were so food limited that they died of starvation.

During the second half of the growing season, predation by juvenile fish may usually explain all of the mortality in the larger classes of the daphnids, resulting in the conclusion that *Daphnia* numbers may be regulated by 0+ fish later in the year. Thus, if juvenile fish biomass is as high as in 1989, the densities of the smaller size-classes of daphnids will be higher than the densities of the larger size-classes of *Daphnia* species. In contrast, when fish densities are lower (1990), larger

size-classes of daphnids will be more dominant. This type of seasonal regulation of *Daphnia* species by fish was also reported by Luecke et al. (1990) in Lake Mendota. The important difference between lakes Mendota and Tjeukemeer is that the main planktivores in Lake Mendota are older (>2+) year-classes of cisco (*Coregonus artedii*) and yellow perch (*Perca flavescens*); these fish were present throughout the year, in contrast to the case with 0+ fish in Tjeukemeer. Predation by cisco and yellow perch, however, also failed to explain the high mortality resulting in the end of the spring maximum densities of *Daphnia* species.

In their study on the annual variation of the food parameter of fish, *a*, Mooij et al. (1994) observed that the year-specific *a* values never differed by more than 20% from the grand mean of the years 1976–1988. The values of *a* calculated in this study (Table 4) for 1990 and 1991 were within this 20% range and hence there were no indications of food-limited growth of fish in these years. However, the values of *a* for the first part of 1989 (before 15 July) were all more than 20% higher than the species-specific grand means. For the second part of 1989 the values of *a* for pikeperch and perch were more than 20% lower than the average value. The *a* values of smelt, bream, and roach in the second half of 1989 were within the normal range. The low *a* values indicate apparent food limitation of the juvenile fish in 1989 after mid July, and could explain our observation that in 1989 more of the total mortality of the larger size-classes seems to be explained by fish predation than in the other years. The food limitation for pikeperch and perch could be explained by the observation that the densities of the preferred zooplankton (*L. kindtii* for pikeperch; copepods for perch) of these fish species were low after mid July compared with the other 2 years.

In conclusion, predation by 0+ fish usually affects population dynamics of *Daphnia* species in Tjeukemeer only in the second half of the year. The mortality that occurs when the population densities decrease in spring is too large to be explained by fish predation. Moreover, the smallest size-class of the daphnids is hardly eaten at all by 0+ fish.

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Filtration dynamics of the zebra mussel, *Dreissena polymorpha*

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Abstract: We quantified the effects of particle type, size, and concentration, mussel size, and temperature on filter-feeding rates of *Dreissena polymorpha* (Bivalvia). Experiments using plastic microspheres yielded reliable filtration estimates when measurements were made prior to the onset of feces production. Once filtered material passed through the gut, filtration of microspheres was much slower than filtration of natural seston. Particles equal to or greater than 1.5 μm in diameter were retained with nearly total efficiency. Clearance rates ($mL \cdot h^{-1}$) were maintained at a maximum level at particle concentrations less than 27 $mg \cdot L^{-1}$. Filtration rates ($mg \cdot h^{-1}$) stabilized at a maximum level above 27 $mg \cdot L^{-1}$. The onset of pseudofeces production, indicating overload of the filtration apparatus, also occurred in the majority of mussels at approximately 27 $mg \cdot L^{-1}$. Filtration per unit gill area was constant regardless of mussel size. Optimal filtration occurred between 14 and 26°C. The filtration Q_{10} (8–20°C) equaled 2.4 and 1.7 for cold- and warm-acclimated mussels, respectively. Similarly, the Q_{10} (8–22°C) of the beat rates of lateral cilia and laterofrontal cirri equaled 2.0. At a common temperature and adjusted for body size, clearance rates of *D. polymorpha* were equal to rates reported for other freshwater bivalves, indicating functional similarity between them.

Résumé : Nous avons quantifié les effets du type, de la taille et de la concentration des particules, de la taille des moules et de la température sur le taux de filtration du bivalve *Dreissena polymorpha*. Des expériences faisant appel à des microsphères de plastique ont donné des estimations fiables de la filtration quand les mesures étaient faites avant le début de la production de fèces. Une fois que le matériau filtré était passé dans l'intestin, la filtration des microsphères était beaucoup plus lente que celle du seston naturel. Les particules de diamètre égal ou supérieur à 1,5 μm étaient retenues avec une efficacité presque totale. La vitesse de circulation ($mL \cdot h^{-1}$) se maintenait à un niveau maximal pour des concentrations de particules inférieures à 27 $mg \cdot L^{-1}$. Les taux de filtration ($mg \cdot h^{-1}$) se stabilisaient à un niveau maximal au-dessus de 27 $mg \cdot L^{-1}$. Le début de l'apparition de pseudofèces, qui indique une surcharge du système de filtration, s'est aussi produit chez la majorité des moules à environ 27 $mg \cdot L^{-1}$. La filtration par unité de surface de branchies était constante quelle que soit la taille des moules. La filtration était optimale entre 14 et 26°C. Le Q_{10} de filtration (8 à 20°C) était de 2,4 et 1,7, respectivement, pour les moules accoutumées à l'eau froide et à l'eau chaude. De même, le Q_{10} (8 à 22°C) du battement des cils latéraux et des cirres latérofrontaux atteignait 2,0. À température courante et après ajustement en fonction de la taille du corps, les taux de circulation de *D. polymorpha* étaient égaux à ceux qui avaient été signalés pour d'autres bivalves d'eau douce, ce qui indique une similarité fonctionnelle.
[Traduit par la Rédaction]

Introduction

Lamellibranch bivalves are enormously successful filter feeders. Where these animals occur in high density, they can substantially alter the trophic dynamics of aquatic ecosystems by filtering suspended particulate material and depositing agglomerations of this material and mucus as feces and pseudofeces (e.g., Cohen et al. 1984). Particle type, size, and concentration as well as mussel size and temperature can greatly affect filtration (see Jørgensen 1990 for recent review). These effects on individuals must be quantified for accurate predictions of a population's effect on an ecosystem.

Dreissena polymorpha, a freshwater lamellibranch native to the Ponto-Caspian region of Europe, was recently introduced into the Great Lakes of North America, from where it subsequently and rapidly spread throughout the New York State Barge Canal and the Mohawk, Hudson, Illinois, Mississippi, Ohio, Tennessee, Cumberland, and Arkansas rivers (New York Sea Grant 1994). Predictions of substantial filtration impact of high-density populations of *D. polymorpha*

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Table 1. Relationship between absorbance (ABS) of 450-nm light and concentration (CON) of seven sizes of microsphere suspensions.

CON	Microsphere diameter (μm)						
($mg \cdot L^{-1}$)	0.5	1.0	1.5	2.0	3.1	4.0	5.1
100.0	1.970	1.838	0.916	0.900	0.702	0.31	0.289
50.0	1.352	1.249	0.489	0.476	0.370	0.162	0.145
25.0	0.752	0.752	0.254	0.249	0.195	0.082	0.074
12.5	0.394	0.395	0.130	0.127	0.105	0.04	0.039
6.3	0.205	0.203	0.067	0.069	0.059	0.023	0.022
3.1	0.110	0.101	0.037	0.037	0.035	0.013	0.011
a	−1.83	−2.47	−1.86	−1.93	−2.69	−0.97	−1.14
b	37.7	40.9	109.9	112.2	145.3	315.0	352.5
r^2	0.982	0.982	0.999	0.999	0.999	0.999	0.999

Note: Regression equation: $CON = a + b(ABS)$.

(MacIsaac et al. 1992) indeed have corresponded to observed changes in Lake Erie seston (Leach 1993). If this species becomes ubiquitously distributed at high density throughout inland rivers, the trophic dynamics of these ecosystems might also be substantially altered (Strayer et al. 1994).

Here we report the results of a series of laboratory studies of factors affecting *D. polymorpha* filtration. Our objectives were to quantify effects of particle type, size, and concentration, mussel size, and temperature on filtration. Quantification of the effects of these factors on *D. polymorpha* filtration allows comparisons with other freshwater and marine species and is required for predictions of trophic consequences of this invasive species in North America.

Material and methods

Mussel collection and handling

All mussels were collected from the upper approach guide wall of the Black Rock Lock in the Black Rock Canal (BRC), a side channel of the Niagara River in Buffalo, New York. Mussels were placed among moistened towels in plastic bags, packed below ice, and shipped overnight to the Vicksburg, Mississippi, laboratory. Mortality during shipment was negligible.

Upon arrival at the laboratory, mussels were transferred to a recirculating stream (activated charcoal filtration) filled with aged tap water maintained at the appropriate acclimation temperature for 2 weeks prior to each experiment. Except in experiments designed specifically to evaluate the effects of mussel size, or unless otherwise indicated, only mussels between 20 and 24 mm shell length were used, to minimize size-related variation. Mussels were not intentionally fed during laboratory acclimation. Prior to filtration measurements, individuals were separated from their byssal attachments to one another, brushed clean, and, unless otherwise noted, kept for approximately 12 h (at their acclimation temperature) in water collected from a turbid inlet into Brown's Lake, Vicksburg, Mississippi, to stimulate feeding.

Filtration and clearance rate measurements

A brief explanation of terms is useful. We use the term filtration rate for the mass of particles captured by, and the term clearance rate for the volume of water passed through, a mussel's gills per unit time. Filtration rate equals ingestion rate only if no pseudofeces are produced. Pseudofeces are filtered particles, agglomerated with mucus, that are periodically ejected back out the inhalant opening to a mussel's mantle cavity prior to ingestion. Although we did not quantify pseudofeces production rate, ingestion rate equals filtration rate minus pseudofeces production rate.

The reduction in suspended particle concentration resulting from mussel clearance was determined from spectrophotometric measurement of the reduction in light absorbance. Most experiments utilized suspensions of neutrally buoyant polymer microspheres (Duke Scientific, Palo Alto, Calif.) in filtered (0.45 μm) tap water. Except in an experiment designed to evaluate the effects of microsphere size on filtration, 2.0 μm diameter microspheres (standard deviation was less than 3% of the mean) were used. Two experiments described below involved natural material (mostly clay with adsorbed bacteria) suspended in the lake inlet water. Coulter counter analysis of this seston indicated that nearly all particles were 1.1–1.5 μm in equivalent spherical diameter. The value of the light absorbance measured directly during filtration of microspheres was converted to concentration using relationships derived for known concentrations (Table 1). Natural particles suspended in the lake inlet water at 2–60 $mg \cdot L^{-1}$ yielded the following relationship of concentration (CON) to absorbance (ABS) of 670-nm light: $CON = (567 \cdot ABS) - 0.61$ ($r^2 = 0.999$; $n = 6$). Light absorbance was measured using a Brinkmann Instruments model 901 dipping-probe colorimeter coupled to a chart recorder. As detailed by Hornbach et al. (1991), this fiber optic device allows continuous monitoring of absorbance decline without removal of water samples.

During filtration measurements, individual mussels were placed on a stainless-steel mesh platform in beakers containing 15–50 mL (depending on mussel size) of a given test suspension. The suspensions were kept well mixed by using a small magnetic stirrer below the mesh platform. Because suspended particles were essentially neutrally bouyant, stirring was so gentle that there was no resuspension of feces or pseudofeces deposited by the mussel during filtration rate measurements. Constant temperature was maintained using a water bath (±0.1°C). The mussels usually began to filter a few minutes after being placed in the test suspension. If after 30 min a mussel had not started to feed, it was replaced. Stirred suspensions without mussels were used as controls to evaluate sedimentation (always negligible).

Mussels were allowed to filter until an approximately 25% reduction in light absorbance was measured. Initial and final light absorbance values were converted to particle concentration using the standard curves described earlier. The mass of material filtered was calculated by multiplying the reduction in concentration by the volume of the test suspension. Division of this quantity by elapsed time (typically 5–20 min) provided an estimate of filtration rate ($mg \cdot h^{-1}$). Clearance rate ($mL \cdot h^{-1}$) was estimated by dividing filtration rate by the mean of the test suspension concentration at the start and end of the filtration period. Each mussel was measured for shell length (SL) and, unless otherwise noted, used for determinations of dry weight (DW, including shell) after the mussel was dried at 70°C to constant weight. Ash-free dry weight (AFDW) was determined after mussels were ashed for 4 h at 550°C.

Particle type effects

Mussels used in this experiment were collected in March 1992 and acclimated at 8°C. A preliminary experiment was conducted to compare filtration of microspheres and natural seston. Filtration rates of individual mussels ($n = 4$, SL range = 24–34 mm) were assessed using a 27 mg·L^{-1} suspension. Mussels were starved for 4 h just prior to this experiment to clear their guts. Using a relatively large volume (approximately 100 mL), filtration was assessed for approximately 3 h. Occasionally, a small volume (1–2 mL) of concentrated suspended particles was added to the test suspensions to reestablish the original particle concentrations. Two filtration estimates were made: one before and one after the first appearance of feces. Typically, between 0.5 and 1 h elapsed prior to the appearance of feces. Mussels used in this preliminary experiment were not used for DW determinations but ranged substantially in size. Consequently, filtration rates were divided by gill area (estimated from regression on SL) to reduce size-related variation.

Particle size effects

Mussels used in this experiment were collected in July 1992 and acclimated at 20°C. Filtration ($n = 8$) was measured for the same mussels sequentially in seven suspensions, each made using microspheres of different diameters. Mussels were kept overnight in water from the Brown's Lake inlet between each treatment. The mean diameter of microspheres in each size class equaled 0.5, 1.0, 1.5, 2.0, 3.1, 4.0, and 5.1 µm. The initial concentration of microspheres in each test ranged from 25 to 33 mg·L^{-1}.

Particle concentration effects

The concentration of suspended solids above which pseudofeces production is initiated was determined by exposing eight mussels in each of three size-classes to five particle concentrations. These mussels were collected in March 1992 and acclimated at 8°C. The SL ranges of small, medium, and large mussels were 9–12, 19–22, and 25–35 mm, respectively. Microsphere concentrations were 5, 13, 27, 43, and 64 mg·L^{-1}. Individuals were placed in nearly filled 50- to 200-mL beakers and allowed to filter for approximately 2 h. Each mussel was observed to determine if pseudofeces had been produced. Pseudofeces (material that has been filtered but expelled back out the inhalant siphon without being ingested) were readily apparent as globular agglomerations of white microspheres and mucus.

Mussels collected in September 1992 and acclimated at 20°C were used in a second experiment to quantify the effects of particle concentration on filtration rate. Rates ($n = 8$, using the same mussels in each treatment) were measured sequentially at six concentrations: 7.4, 12.2, 27.4, 37.2, 49.7, and 83.5 mg·L^{-1}.

Mussels collected in March 1993 and acclimated at 20°C were used in a third experiment to assess particle concentration effects on siphonal openings and shell gape. Measurements were made of mussels exposed to Brown's Lake inlet water. The suspended solids concentration of the lake inlet water was 62 mg·L^{-1}. This water was diluted (using inlet water passed through a 0.45-µm filter) to achieve concentrations of 31, 15.5, and 8 mg·L^{-1}. Starting with the lowest concentration, the same group of eight mussels was sequentially exposed to the four concentrations. Mussels were acclimated to each test concentration for 1 h prior to measurements. Mussels were anchored on a piece of modeling clay on the bottom of nearly filled 250-mL beakers such that the openings of their inhalant siphons faced upward and were parallel to the bottom of the beakers. Once mussels had opened and filtered the gently stirred suspension for a few minutes, siphon aperture area (mm^2) and shell gape (mm) were measured from a single magnified video image using Video Image IPPLUS software. The mussels were then repositioned and the exhalant siphon aperture was assessed in the same manner.

Temperature effects

Mussels were collected in September 1992 and acclimated at 8 and 20°C for 2 weeks. The filtration rates of eight mussels from each acclimation group were measured sequentially at each of the following test temperatures: 8, 14, 20, 26, and 32°C. A microsphere concentration of 12 mg·L^{-1} was used.

A separate experiment was conducted to evaluate temperature effects on the beat rate of lateral cilia and laterofrontal cirri of the gill. Mussels were collected in May 1993 and acclimated at 8, 13, 18, and 22°C for 2 weeks. A gill fragment was excised from each of eight mussels per acclimation group, mounted on a glass slide, and observed at 100× magnification. Within 5 min of excision, a videotape was made of beating cilia and cirri. The tape was played back at 0.1–0.2 times actual speed to allow the beat frequency of cilia and cirri to be measured. Frontal cilia could not be resolved sufficiently to determine their beat frequency.

Mussel size effects

Mussels were collected in July 1992 and acclimated at 20°C. Filtration rates for a total of 10 mussels (SL range 13–23 mm) were measured using microspheres at an initial concentration of 12 mg·L^{-1}. These mussels were sacrificed for measurement of SL, DW, and AFDW. A separate group of 18 mussels (SL range 13–34 mm, enlarged to support analysis of the experiment on particle type effects) was subsequently used for determinations of gill area (GA) in relation to SL to support analysis of GA effects on filtration. GA was measured by excising the inner right demibranch, laying it flat on a glass slide, capturing a magnified video image, and estimating the area of the upward-facing lamella. This area was then multiplied by eight (two lamellae per each of four demibranchs) to provide an estimate of GA.

Statistical analyses

Analysis of variance (ANOVA) was used to determine the significance of treatment effects on the various filtration parameters measured. Data were logarithmically transformed when necessary to obtain homogeneous distribution of variance. Two-way ANOVA was used to test the significance of single factor and interactive effects of particle type and time (relative to the onset of feces production) as well as test and acclimation temperatures. Tukey's honestly significant difference test was used to test the significance of differences among treatment means.

Fig. 1. The effects of microsphere diameter on filtration rates of *Dreissena polymorpha* (±SE). If not shown, SE bars are within the solid circle (representing the mean). Means designated by the same letter are not significantly different ($p > 0.05$) according to Tukey's HSD test. The broken line was fit to treatment means using the Statistica (1994) distance-weighted least-squares procedure.

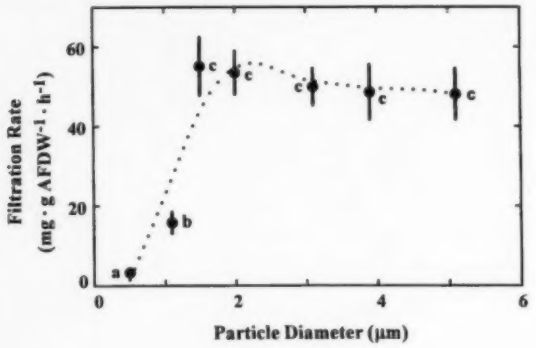


Table 2. Comparison of filtration rates (FR), expressed per unit gill area, of *Dreissena polymorpha* exposed to 27 mg·L^{-1}.

Particle type	Time relative to onset of feces production	FR (mg·cm^{-2}·h^{-1})		HSD grouping
		Mean	SE	
Natural	Before	0.282	0.039	A
Natural	After	0.224	0.021	A
Microspheres	Before	0.224	0.035	A
Microspheres	After	0.033	0.015	B

Note: Differences among treatment means ($p < 0.005$) were tested using Tukey's honestly significant difference (HSD) test.

Results

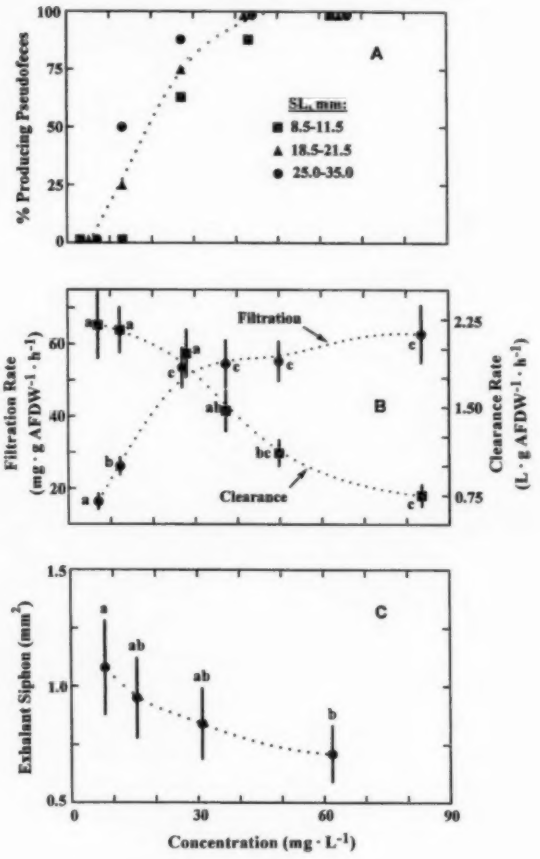
Particle type effects

After time for passage of material through the gut, mussels greatly reduced their filtration of plastic microspheres but not natural seston (Table 2). Filtration of natural seston was reduced by only 21% after the onset of feces production. In contrast, filtration of microspheres was 87% less after than before the onset of feces production. Significant single factor effects on filtration were observed for particle type ($F_{[1,12]} = 20.7$; $p < 0.001$) and time (i.e., before versus after onset of feces production) ($F_{[1,12]} = 23.0$; $p < 0.001$). The interactive effect of particle type and time was highly significant ($F_{[1,12]} = 15.7$; $p < 0.005$). All measurements subsequently reported here using microspheres were made prior to the onset of feces production.

Particle size effects

Filtration was significantly affected by microsphere diameter ($F_{[6,49]} = 61.9$; $p < 0.001$). Filtration increased 4.8 times as microsphere diameter increased from 0.5 to 1.0 µm and 3.5 times from 1.0 to 1.5 µm (Fig. 1). Maximum filtration was achieved using 1.5 µm diameter microspheres and did not vary significantly as microsphere diameter increased from 1.5 to 5.1 µm. The retention efficiency of each particle size-class was estimated by dividing filtration of a given size-class by maximum filtration among all size-classes. Thus computed, retention efficiency equaled just 6 and 29% for microspheres of 0.5 and 1.0 µm diameter and was uniformly high, ranging from 87 to 100% for all larger microspheres.

Fig. 2. The effects of particle concentration on pseudofeces production (A), filtration and clearance rates (B), and exhalant siphon area (C) of *Dreissena polymorpha*. SE bars are shown in B and C; means designated by the same letter are not significantly different ($p > 0.05$) according to Tukey's HSD test. The broken lines were fit to treatment means using the Statistica (1994) distance-weighted least-squares procedure.



Particle concentration effects

The majority of mussels, regardless of size, produced pseudofeces at 27 mg·L^{-1} (Fig. 2A). At a lower particle concentration of 13 mg·L^{-1}, some of the large and medium mussels produced pseudofeces. At the lowest concentration of 5 mg·L^{-1}, no mussels produced pseudofeces whereas at the highest concentration of 64 mg·L^{-1}, all mussels produced pseudofeces.

Filtration increased significantly as a function of particle

Table 3. Relationships of clearance rate (CR, mL·h^{-1}) and gill area (GA, mm^2) to ash-free dry weight (AFDW, mg) and dry weight (DW, mg) and of GA, AFDW, and DW to shell length (SL, mm).

Y	*X*	*a*	*b*	*r*	*n*
CR	AFDW	9.5	0.48	0.917	10
CR	DW	108.7	0.63	0.979	10
GA	AFDW	40.3	0.53	0.964	9
GA	DW	508.9	0.62	0.977	10
GA	SL	1.0	1.90	0.960	18
AFDW	SL	2.89×10^{-3}	3.28	0.992	10
DW	SL	3.81×10^{-2}	3.04	0.913	10

Note: Regression equation: $Y = a(X)^b$.

concentration ($F_{[5,42]} = 22.2$; $p < 0.001$). Filtration increased by more than three times as particle concentrations increased from 7 to 27 mg·L^{-1} (Fig. 2B). However, particle concentrations above 27 mg·L^{-1} did not produce significant increases in filtration rate. Because the 2.0 µm diameter microspheres used to evaluate these concentration effects on filtration were retained with nearly total efficiency (Fig. 1), filtration data obtained using this particle size provided accurate estimates of clearance.

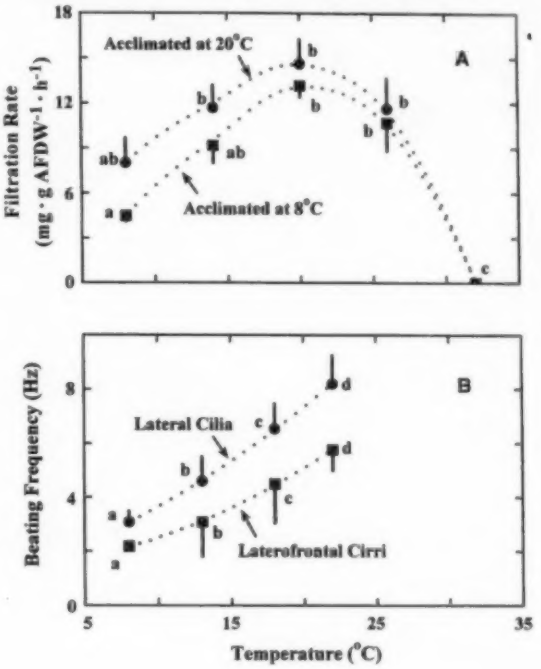
Clearance decreased significantly with increased concentration ($F_{[5,42]} = 14.4$; $p < 0.001$) (Fig. 2B). However, a comparison among treatment means showed significant differences only at concentrations greater than 27 mg·L^{-1}. Clearance decreased 62% as concentration increased from 27 to 84 mg·L^{-1}.

Particle concentration significantly affected the size of the exhalant siphon ($F_{[3,32]} = 5.88$; $p < 0.004$) but not the inhalant siphon ($F_{[3,32]} = 0.0351$; $p > 0.99$) or shell gape ($F_{[3,32]} = 0.350$; $p > 0.78$). The average aperture of the exhalant siphon decreased 34% as particle concentration increased from 8 to 62 mg·L^{-1} (Fig. 2C). In addition, observation of videotaped mussels revealed that the exhalant siphon was much more active than the inhalant. The inhalant siphon, once expanded, rarely changed position or size. In contrast, muscles of the exhalant siphon contracted intermittently, which shortened the siphon briefly and greatly reduced its aperture. Siphon area was measured in relation to particle concentration during sustained periods of siphon expansion.

Temperature effects

Filtration was significantly affected by both acclimation temperature ($F_{[1,70]} = 4.35$; $p < 0.05$) and test temperature ($F_{[4,70]} = 10.3$; $p < 0.001$) but not by their interaction ($F_{[4,70]} = 0.564$; $p > 0.64$). Both cold- and warm-acclimated mussels showed increased filtration rates from 8 to 20°C and decreased filtration rates from 20 to 32°C (Fig. 3A). Because no temperature treatments were used between 20 and 26°C, the filtration rate may have been maximal at a temperature slightly greater than 20°C. Filtration was not detectable at 32°C for mussels at either acclimation temperature, indicating extreme disruption of normal physiological function. Acclimation temperature had a consistent minor effect on filtration; filtration at a given test temperature was always slightly lower for cold- than for warm-acclimated mussels (Fig. 3A). The filtration Q_{10} from 8 to 20°C equaled 2.4 and 1.7 for cold- and warm-acclimated mussels, respectively.

Fig. 3. The effects of temperature on the filtration rate of *Dreissena polymorpha* (±SE) (A) and the beat frequency of lateral cilia and laterofrontal cirri of excised gills (±SE) (B). For the filtration study, mussels were acclimated at 8 and 20°C for 2 weeks and tested at 8, 14, 20, 26, and 32°C. For the study of the beat frequency of gill cilia and cirri, mussels were acclimated for 2 weeks and tested at 8, 13, 18, and 22°C. If not shown, SE bars are within the solid circle (representing the mean). Means designated by the same letter are not significantly different ($p > 0.05$) according to Tukey's HSD test. The broken lines were fit to treatment means using the Statistica (1994) distance-weighted least-squares procedure.

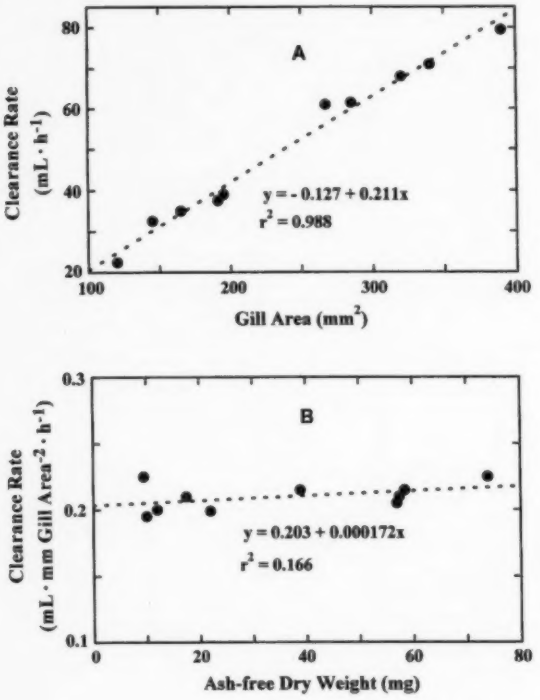


The beat frequency of both lateral cilia ($F_{[3,28]} = 675$; $p < 0.001$) and laterofrontal cirri ($F_{[3,28]} = 204$; $p < 0.001$) increased significantly with temperature from 8 to 22°C (Fig. 3B). The estimated Q_{10} of the beat frequency of both cilia and cirri equaled 2.0 over this temperature range and closely matched the average filtration Q_{10} (2.05) of cold- and warm-acclimated mussels. It is also noteworthy that among all acclimation–test temperature combinations the ratio of the beat rate of laterofrontal cirri to that of lateral cilia ranged only from 0.69 to 0.71. Thus, the functional relationship between cirri and cilia was independent of temperature.

Mussel size effects

Particle clearance increased as a positive exponential function of mussel body mass (Table 3). However, clearance rate was a strict linear function of gill area (Fig. 4A). Variation in clearance related to body mass was totally compensated for if clearance was divided by gill area (Fig. 4B). It is clear from these

Fig. 4. Clearance rate of *Dreissena polymorpha* expressed per individual in relation to gill area (A) and per unit gill area in relation to ash-free dry weight (B).


A
Clearance Rate (mL · h⁻¹)
y = - 0.127 + 0.211x
r² = 0.988
Gill Area (mm²)
B
Clearance Rate (mL · mm Gill Area⁻² · h⁻¹)
y = 0.203 + 0.000172x
r² = 0.166
Ash-free Dry Weight (mg)

results that gill area and clearance rates have virtually identical relationships to body mass (Table 3).

Discussion

Particle type effects

Previous studies indicated that natural seston is filtered faster than inert, non-nutritious particles (Dral 1967; Yonge and Campbell 1968; Morton 1971; Higgens 1980). Nevertheless, plastic microspheres have been used extensively in bivalve filtration studies (e.g., Jørgensen et al. 1984; Burky et al. 1985; Way 1989; Way et al. 1990; Vanderploeg et al. 1995). However, as demonstrated in the present study, decreased filtration of plastic microspheres is not problematic until these materials have had time to pass through the digestive system. Way (1989) reported similar results for *Musculium transversum*, showing no significant difference in the short-term filtration rate of microspheres versus of *Chlorella* sp. of essentially the same diameter. If filtration measurements are made prior to the onset of feces production, microspheres provide a reliable, easy, and precise method of evaluating the effects of particle size and concentration on filtration.

Particle size effects

Dreissena polymorpha has a gill approximately as retentive as any bivalve and more retentive than most. Our results indicate that *D. polymorpha* retains plastic microspheres 0.5 and 1.1 μm in diameter with efficiencies of 6 and 29% and microspheres 1.5 μm in diameter and larger with nearly 100% efficiency. Similarly, Jørgensen et al. (1984) reported greater than 90% retention by *D. polymorpha* of spherical algae and plastic microspheres greater than 1.5 μm in diameter, although results were not reported for smaller particle sizes. In contrast, studies of *D. polymorpha* by Sprung and Rose (1988), using pure cultures of essentially spherical algae ranging in average diameter from 1 to 30 μm, indicated a less retentive gill. Although some algae as small as 0.7 μm were retained, complete retention was of algae greater than 5 μm in diameter.

Comparisons can be made with a few other freshwater species. Complete retention of microspheres as small as 1.0 μm in diameter was reported for both *M. transversum* (Way 1989) and *Corbicula fluminea* (Way et al. 1990). *Musculium transversum* is slightly smaller and *C. fluminea* slightly larger than *D. polymorpha*. The filtration of small particles by the relatively massive native North American unionid *Lampsilis radiata siliquoidea* was studied by Vanderploeg et al. (1995). They observed complete retention of natural seston with equivalent spherical diameters of 1.5 μm or greater. Jørgensen et al. (1984) showed that *Unio pictorum* and *Anodonta cygnea* retained particles 1.5 μm in diameter with only 40–60% efficiency and did not achieve essentially complete retention until particle diameter reached 3–5 μm. Considerably more information has been published for marine bivalves, with thresholds for near-complete particle retention averaging approximately 4 μm and ranging from 2 to 7 μm (Jørgensen 1955; Møhlenberg and Riisgård 1978; Riisgård 1988). Thus, available data indicate that freshwater bivalves are slightly more effective than marine bivalves in retaining small particles.

Picoplankton (<3 μm in equivalent spherical diameter (ESD), very fine detritus, and bacteria are likely to be important in the diets of many freshwater bivalves. Fahnenstiel and Carrick (1992) showed that phytoplankton <3 μm ESD account for 40% of phototrophic production in lakes Michigan and Huron. Vanderploeg et al. (1995) showed that suspended particles <3 μm ESD comprised 35–49% of total seston in Lake St. Clair. It is worth recapitulating that *D. polymorpha* is not unique among freshwater bivalves in its ability to filter very small particles. Changes in the trophic dynamics of aquatic ecosystems caused by *D. polymorpha* are more likely simply to reflect a tremendous increase in bivalve filtration wherever this species is widely distributed at high density, such as Lake Erie (MacIsaac et al. 1992).

Particle concentration effects

The clearance rate of bivalves tends to decrease as particle concentration increases (e.g., Loosanoff and Engle 1947; Loosanoff 1961; Davids 1964; Stickney 1964; Ali 1970; Foster-Smith 1975; Schulte 1975; Widdows et al. 1979; Wilson 1983). However, as pointed out by Winter (1973), important facets of independence add detail to this general inverse relationship. Clearance remains high over a range of low concentrations, and filtration remains high over a range of high concentrations. Such a pattern exactly matches our results with *D. polymorpha* as well as previous studies by both Walz (1978) and Sprung and Rose (1988). In the present study, the limiting concentration (cf. Walz 1978) for simultaneous clearance reduction and filtration stabilization was approximately

27 mg·L^{-1} and corresponded closely with the concentration at which pseudofeces production was initiated by a majority of mussels. This close correspondence strongly suggests physiological regulation of filtration in response to particle concentration (Bayne et al. 1993). An alternative view is that a decline in the clearance rate fortuitously leads to filtration stabilization but does not necessarily imply physiological regulation (Jørgensen 1990).

The limiting concentration of approximately 27 mg·L^{-1} that we observed for *D. polymorpha* is similar to that reported for other small bivalves. Fuji (1979) observed that *Corbicula japonica* consistently produced pseudofeces at particle concentrations greater than 20 mg·L^{-1}. Way et al. (1990) reported pseudofeces production by *Corbicula fluminea* at particle concentrations equal to or greater than 17 mg·L^{-1}. The limiting concentration for *Musculium transversum* has been estimated to equal 26 mg·L^{-1} (Way 1989). Hornbach et al. (1984) reported that pseudofeces were produced by *Sphaerium striatinum* at particle concentrations greater than 20 mg·L^{-1}. Walz (1978) observed a lower limiting concentration for *D. polymorpha*, although the pattern of response was the same as we observed. Using cultured diatoms as food, Walz observed a simultaneous reduction of clearance rate and stabilization of pseudofeces production, as a percentage of filtration rate, at diatom dry weight concentrations equal to or greater than 7 mg·L^{-1}.

The rate at which water is drawn through the mantle cavity theoretically can be altered in response to particle concentration by changing the size of the inhalant and exhalant siphons or water passages through the gill. Jørgensen et al. (1990) suggested a simple mechanism by which reduced shell gape decreases siphonal openings as well as interfilamentar space and thus increases resistance to flow through the bivalve pump. This mechanism did not apply to *D. polymorpha* in the present study. Neither the size of the inhalant siphon nor that of the shell gape responded to particle concentration. However, exhalant siphon area was reduced with increased particle concentration. A similar restriction of exhalant siphon area with increased particle concentration was observed in *Cerastoderma edule* and *Venerupis pullastra* by Foster-Smith (1975). Presumably, reducing the exhalant siphon area at higher particle concentrations increases back-pressure in the mantle cavity pump and either contributes to or accounts for reduced clearance.

Mussel size effects

The filtration rate (FR) typically varies in relation to body size, following the power function $FR = a \cdot (W)^b$, where W is mussel weight. On theoretical grounds, it has been argued that the b value of this relationship should equal 0.75 (Jørgensen 1976). Winter (1978) concluded that values lie between 0.66 and 0.82, in close agreement with exponents for rates of oxygen consumption. In practice, b values range more widely, from approximately 0.4 to 1.0 (e.g., Møhlenberg and Riisgård 1979; Meyhöfer 1985; Riisgård 1988). Values of 0.48 (AFDW) and 0.63 (DW) were observed in the present study. The covariance of filtration rate and weight confuses comparisons of filtration rates both within and among studies. Thus, it is noteworthy that, on a per area basis, gills of large and small *D. polymorpha* clear water at the same rate (Fig. 4B). Such strict proportionality between filtration rate and gill area has also been observed in marine lamellibranch species (Møhlenberg and Riisgård 1979; Meyhöfer 1985).

Table 4. Clearance rates (CR) at 15°C of freshwater bivalves spanning virtually the entire weight range (AFDW) of this faunal group.

Species	AFDW	CR (mL·h^{-1})	Source
Musculium transversum	5	15	Way 1989 (Fig. 1 therein)
	10	30	
Corbicula fluminea	50	60	Way et al. 1990 (Fig. 1 therein)
	150	150	
	300	290	
Dreissena polymorpha	57	74	Present study
Lampsilis radiata siliquoidea	2020*	960	Vanderploeg et al. 1995 (Table 4 therein)

*Assumes AFDW = 0.95 DW (without shell).

Not only is filtration limited by gill area, but there is considerable evidence that gill development depends on environmental conditions affecting filtration. Franz (1993) observed that *Geukensia demissus* living low in the intertidal zone have proportionately smaller gills than those living high in the intertidal zone. He suggested that reduced submersion time of high intertidal mussels dictated larger gills and thus an increased filtration rate. Similarly, bivalves from habitats characterized by relatively low concentrations of suspended solids develop proportionately larger gills, and, presumably, greater maximum filtration rates, than individuals from habitats characterized by high concentrations of suspended solids (Theisen 1982; Payne et al. 1995).

Routine measurement of gill area is recommended for bivalve filtration studies. Potentially adaptive aspects of gill development are more likely to be recognized with such measurements. In addition, within populations (at least), filtration rates are relatively constant if expressed per unit gill area. Such constancy of rates can be used advantageously in analyses of factors, other than mussel size, that affect filtration.

Temperature effects

Dreissena polymorpha filters maximally at an intermediate temperature, regardless of acclimation temperature. Walz (1978) reported optimum filtration at 15°C. In the present study, optimum filtration occurred between 14 and 26°C. These results agree with the view that *D. polymorpha* is physiologically stressed at moderately high temperature (>25°C) (Schneider 1992 and references therein; Aldridge et al. 1995). Although this introduced species has established naturally recruiting populations in the lower Mississippi River (Dr. Ken Brown, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803, U.S.A., personal communication; J. Lei, B.S. Payne, S.Y. Wang, unpublished data), it remains to be seen how mussels respond to stressful summer temperatures of 29–31°C.

At suboptimal temperatures, there was a clear positive effect of temperature on filtration regardless of acclimation

temperature (see also Morton 1971; Hinz and Scheil 1972; Stanczykowska et al. 1976; Walz 1978). In contrast, Reeders et al. (1989) concluded that filtration by a lake population of *D. polymorpha* in the Netherlands did not vary seasonally with changes in water temperature from 7 to 20°C. The filtration Q_{10} (1.5–2.4) measured in the present study closely coincided with the estimated Q_{10} (2.1) of the beat frequency of both lateral cilia and laterofrontal cirri. Similar Q_{10} values for the beat frequency of lateral cilia have been reported for marine bivalves (Aiello 1960; Stefano et al. 1977; Jørgensen et al. 1990). The similar response of the lateral cilia beat and the filtration rate to temperature is concordant with the traditional view of a causal relationship (e.g., Dral 1967; Hildreth 1976; Winter 1978; Bayne and Newell 1983). However, an alternative recently suggested by Jørgensen et al. (1988) on the basis of studies of *Mytilus edulis* is that water viscosity, not cilia beat rate, is responsible for the temperature-related variation in filtration rate. Applying this idea to the present study, we estimated that water viscosity decreased approximately 30% with a temperature increase of 8–18°C. Both the lateral cilia beat rate and the filtration rate of *D. polymorpha* increased approximately 100%.

Neither our experimental design nor that of Jørgensen et al. (1988) distinguishes between the effects of viscosity and lateral cilia beat on filtration rate. Only temperature is experimentally controlled, and this variable leads to changes in both cilia beat and viscosity. However, applying Ockham's razor, the similar magnitude of the temperature response of both the lateral cilia beat rate and the filtration rate supports the traditional view of a causal link.

Interspecific similarity of filtration

Clearance rates of *D. polymorpha* are equal to those reported for freshwater bivalves, if considered at a single temperature and adjusted for body size. Table 4 summarizes clearance rate estimates at 15°C for freshwater bivalves ranging from very small (*Musculium transversum*) to large (*L. radiata siliquoidea*). All rates were measured using particles retained with high efficiency at concentrations associated with maximum filtration. Using data in Table 4, $CR = 5.06 \cdot (AFDW)^{0.686}$ ($r^2 = 0.998$). This b value approximates the theoretical expectation of 0.75 (Jørgensen 1976).

Other aspects of filtration by *D. polymorpha* are also basically similar to filtration by other species. Small particle retention by *D. polymorpha* resembles that by other freshwater species. In addition, responses to particle concentration follow a general pattern that applies to most bivalves (Winter 1973). Such functional similarities reflect underlying structural similarities of the filter-feeding apparatus and should facilitate predictions of trophic consequences of invading populations of *D. polymorpha*. Accurate predictions will require not only quantification of factors affecting filtration, but also detailed information on the size demography, distribution, and density both of bivalves (e.g., Payne and Miller 1989; MacIsaac et al. 1992; Miller and Payne 1993; Strayer et al. 1994) and of seston (e.g., Fahnenstiel and Carrick 1992; Vanderploeg et al. 1995).

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Dispersion and mortality of a population of sea scallop (*Placopecten magellanicus*) seeded in a tidal channel

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Abstract: Ten thousand scallops (*Placopecten magellanicus*) from 4 to 26 mm shell height were released in 40 m^2 of an 8 m deep tidal channel in Lunenburg Bay, Nova Scotia, Canada. Their survival, distribution, and potential predators were monitored during the 13 months following release in November 1990 using quadrat and video surveys. Both dispersion and mortality were rapid during the first 2 weeks, when predation by crabs and starfish was estimated to have killed about one half of the seeded population. The rate of loss decreased markedly over the following winter and spring. Dispersion of seeded animals increased again through summer and autumn, producing a final scallop density of about 2 m^{-2} (twice that of the natural population), covering more than 3500 m^2 of seabed. This expansion was accompanied by little further mortality, and the final estimate of survivors in the expanded survey area was 40%. Scallop displacement was directional, but the mean vector did not match that of the dominant, near-bed water currents. The average growth (shell height increment) of surviving scallops was 35 mm in 13 months. Our results demonstrate the potential for ecologically viable bottom culture of the species in coastal Nova Scotia.

Résumé : Dix mille pétoncles (*Placopecten magellanicus*) mesurant de 4 à 26 mm de hauteur de coquille ont été libérés dans une zone de 40 m^2 d'un chenal à marée de 8 m de profondeur, dans la baie de Lunenburg (Nouvelle-Écosse, Canada). Nous avons surveillé leur survie, leur distribution et leurs prédateurs potentiels pendant les 13 mois qui ont suivi leur déversement en novembre 1990, en faisant des relevés sur des quadrats et en prenant des enregistrements vidéo. La dispersion et la mortalité ont été rapides pendant les 2 premières semaines, et on estime que pendant cette période la prédation par les crabes et les étoiles de mer a tué à peu près la moitié de la population ensemencée. Le taux de perte a baissé de façon notable pendant l'hiver et le printemps suivants. La dispersion des animaux a augmenté de nouveau pendant l'été et l'automne, pour aboutir à une densité d'environ 2 m^{-2} (deux fois celle de la population naturelle), les pétoncles ensemencés couvrant plus de 3500 m^2 de fond. Cette expansion ne s'est accompagnée que d'une faible mortalité supplémentaire, et on estime à 40% le pourcentage final de survivants dans la zone élargie. Le déplacement des pétoncles était orienté, mais le vecteur principal ne correspondait pas à celui des courants dominants près du fond. La croissance moyenne (accroissement de la hauteur de la coquille) des pétoncles survivants a été de 35 mm en 13 mois. Nos résultats démontrent le potentiel d'une pectiniculture écologiquement viable sur le fond dans les eaux côtières de Nouvelle-Écosse.
[Traduit par la Rédaction]

Introduction

The fishery for the sea scallop (*Placopecten magellanicus* Gmelin, 1791) is one of Atlantic Canada's most valuable (Sinclair et al. 1985). Depletion of coastal stocks has greatly reduced the viability of the capture fishery at many inshore sites (Caddy 1989), leading to increased interest in stock enhancement and aquaculture (Naidu and Cahill 1986; Dadswell and Parsons 1991).

Two common methods of scallop culture have contrasting trade-offs between yields and production costs. Suspended culture, either in pearl nets or by ear-hanging, generally results in good survival and growth rates of postsettlement juveniles (MacDonald 1986), but requires a large investment of capital and labor. Bottom culture entails release of either wild or hatchery-reared spat directly to the seabed (seeding), for harvest by conventional means (diving, dragging) following a grow-out period. The method generally trades reduced survival and growth for savings of capital and labor costs. It has been applied successfully to several species of scallops elsewhere (e.g., Ventilla 1982; Dao et al. 1994; Bull 1989; Goshima and Fujiwara 1994), but has not been used in the commercial culture of *P. magellanicus*, even though analysis suggests it to be the only method that offers a reasonable economic return on investment (Wildish et al. 1988; Couturier

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1990). Bottom seeding may be used to enhance natural stocks as well as to culture scallops in restricted leaseholds.

Mortality and emigration of animals during the extended growth period of *P. magellanicus* (3 to 5 years from settlement to harvest) are two basic processes limiting yields from both wild and bottom-cultured populations. Suspended culture controls both types of loss by restricting animals' movement and keeping them away from predators, such that morbidity from other causes (e.g., disease, water quality) becomes important (Dupouy 1983; Parsons and Dadswell 1992; Emerson et al. 1994). In bottom culture it is difficult to differentiate between emigration of juvenile scallops from the seeded area and mortality within it (e.g., Bull 1989). Because postsettlement movement and mortality strongly influence the distribution and abundance of scallops in natural populations (Orensanz et al. 1991), quantification of these processes in seeded populations of juvenile *P. magellanicus* across a range of natural environments has ecological as well as economic relevance.

Predators of scallops include starfish, crabs, gastropods, and fish (e.g., Caddy 1968; Kohler and Fitzgerald 1969; Morgan et al. 1980; Volkov et al. 1983; Minchin 1992). Starfish and crabs in particular are recognized as causing significant mortality in both natural and cultured populations of *P. magellanicus* (Dickie and Medcof 1963; Medcof and Bourne 1964; Elner and Jamieson 1979; Dupouy 1983; Barbeau et al. 1994). Large scallops are less susceptible to predation than small ones, depending on the type of predator involved (e.g., Elner and Jamieson 1979; Barbeau and Scheibling 1994*a*), and animals larger than 70 mm shell height (3- to 4-year-old animals in the case of *P. magellanicus*) are considered able to resist attack by many predators (Brand 1991; Orensanz et al. 1991). Well endowed with sensory organs, scallops exhibit behavioral responses to predator attack such as escape swimming (Brand 1991) and migration to local refugia (Smith et al. 1988; Pohle et al. 1991). The distributions of *P. magellanicus* and its predators are correlated over large areas of offshore seabed (Thouzeau et al. 1991), but the behavioral and numerical responses of predator populations to local increases in juvenile scallop abundance (e.g., aggregation of starfish following seeding, Volkov et al. 1983) have not been studied and are particularly relevant to bottom culture.

Juvenile *P. magellanicus* are competent swimmers (Caddy 1968; Dadswell and Weihs 1990; Manuel and Dadswell 1991). Swimming is a metabolically expensive activity (Thompson et al. 1980; de Zwaan et al. 1980) that may be initiated as an escape from a predator, or as an attempt to migrate from undesirable habitat. Swimming trajectories may be oriented (i.e., directional migration, Minchin 1989) or random, in which case displacement patterns may reflect the hydrodynamic regime (Moore and Marshall 1967; Posgay 1981; Caddy 1989). Tagged *P. magellanicus* larger than 60 mm shell height move as much as 10 km/year on Georges Bank (Posgay 1981; Melvin et al. 1985). The dispersion of smaller animals used in bottom culture may also be substantial (Carsen et al. 1995), although Parsons et al. (1992) reported net displacements of only a few metres in 3 months. Detailed, in situ measurements of spatial and temporal variation in the dispersion of seeded scallops are essential for successful management of scallop culture.

As part of a major research program aimed at determining the ecological feasibility of aquaculture and stock enhancement of *Placopecten magellanicus* in eastern Canada (Anonymous 1993), we followed the spatial and temporal trajectories of populations of juveniles seeded in natural benthic habitats. The results will be used to select suitable aquaculture sites, to optimize seeding and grow-out methods, and for cost–benefit analyses of production enhancement strategies. The costs of large-scale field experiments require pilot studies to optimize designs and sampling techniques. In the pilot experiment described here, we released more than 10 000 juvenile scallops in a shallow tidal channel and followed their dispersion, survivorship, and growth over a 13-month period.

Specific objectives of the study were as follows: (*i*) to plot the trajectory of the seeded population in space and time following release in relation to the biotic and abiotic features of the environment, (*ii*) to estimate the losses from the population resulting from dispersion and predation in relation to the spatial and temporal patterns in abundance of various predators and their scallop prey, and (*iii*) to estimate the survivorship and individual growth rates in the seeded population after 1 year as measures of scallop production enhancement in a natural habitat.

Materials and methods

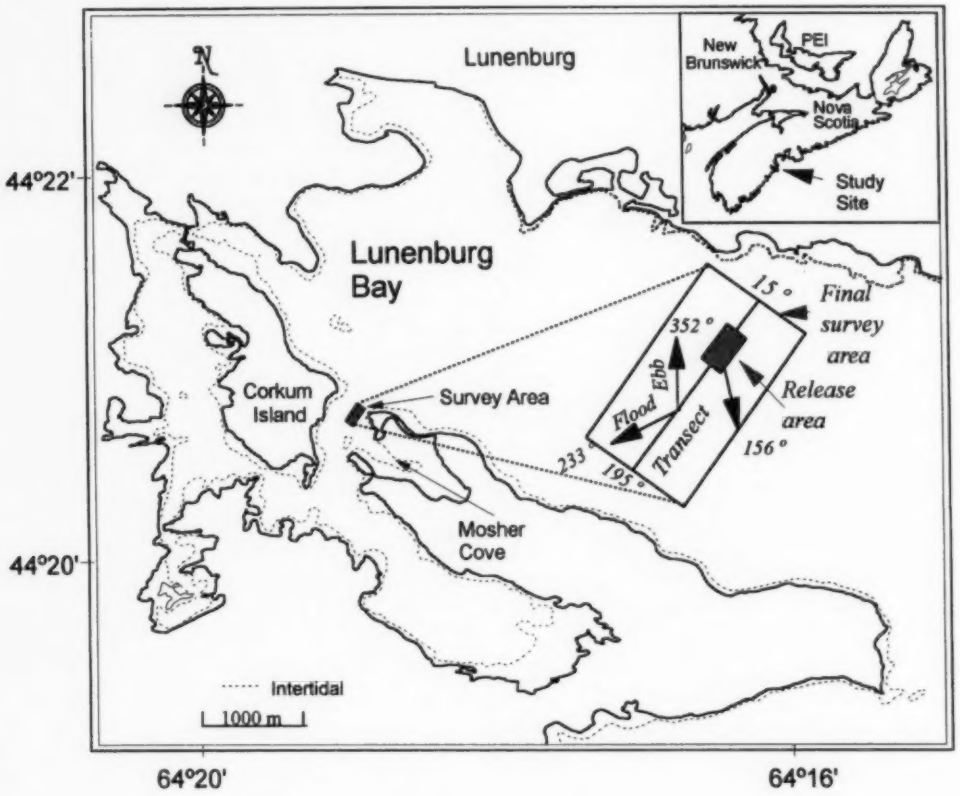
Study site

Corkum Island Channel (Fig. 1) connects two shallow basins (total area approx. 2.8 km^2) with the southwest portion of Lunenburg Bay, Nova Scotia, Canada. The channel is approximately 1 km long and 0.35 km wide, with an average low tide depth of 8.0 m in the centre. The seabed grades from large cobble with kelp in the centre, through shale gravel and shell fragments, to mud and silt with sea grass at the channel margins. A shallow (≅1.0 m deep) basin (Mosher Cove) extends laterally from the southeast side of the channel. The study site (44°20.5′N, 64°18.9′W) is located in an area of uniform depth (7.5 m at low tide) and substratum (small cobbles and shell fragments mixed with mud) on the northeast side of the channel.

Extensive meteorological, hydrological, and hydrodynamic measurements are available for the area (Sturley and Leal 1991; Sturley 1992). Semidiurnal tides (M2 tidal period of 12.42 h) with a mean amplitude of 1.2 m dominate flow through the channel as the shallow basins fill and empty. Along-channel current velocities (vertically averaged over the bottom 0.6 m of the water column at two locations within the study site) average 0.21 $m \cdot s^{-1}$ at 352°T (true compass bearing) on the ebb, and 0.16 $m \cdot s^{-1}$ at 233°T on the flood, with peak speeds reaching 0.6 $m \cdot s^{-1}$ on the ebb tide. An oscillation with a period of approximately 1 h is a consistent feature of the flow, with a velocity capable of halting or reversing the tidal flow. At the centre of the study site, the mean axes of the ebb and flood flows are displaced by approximately 30° to the north and west of the channel axis, respectively (Fig. 1).

Daily mean seawater temperatures near the seabed at the study site ranged from –1.3 to 16.4°C during the 13 months. Semidiurnal temperature variations were often substantial, exceeding 4°C during the autumn and spring. Salinity ranged from 29.1 to 31.4 parts per thousand (ppt) during this period,

Fig. 1. Chart of the study site in Lunenburg Bay, Nova Scotia, showing the channel east of Corkum Island, the layout of the survey area, and the 40-m^2 release area in which scallops were seeded in November of 1990. The along-channel orientation of the main sampling transect, the mean tidal current vectors, and the mean vector of movement of the seeded scallop population (156°) during the 13-month experiment are indicated with arrows (true compass bearings).



with semidiurnal variations of approximately 1.0 ppt during spring tides.

Preseeding survey

The distribution and abundance of scallops and potential predators were measured using self-contained underwater breathing apparatus (SCUBA) during the day prior to seeding (November 19, 1990), in four 25 × 1.0 m belt transects laid parallel to the main axis of the channel. Two transects ran through the centre of the area to be seeded with scallops, and the other two were located 10 m towards the eastern shore. All scallops and scallop valves in three size-classes (5–30, 30–100, and >100 mm as measured against a diver-held scale) were counted in contiguous 0.25-m^2 quadrats along each transect, as were all potential predators of scallops (e.g., starfish, crabs, snails, fish).

Scallop handling and seeding

Juvenile *Placopecten magellanicus*, ranging from 4 to 26 mm shell height, were purchased from a commercial hatchery (Fisheries Resource Development Ltd.) on November 19, 1990. The animals were the progeny of a single, captive spawning of adults collected from Mahone Bay (adjacent to Lunenburg Bay). The scallops were transported in coolers directly from holding facilities in Mahone Bay to the study site. There they were counted, sorted into groups of 400, and suspended in pearl nets in the channel near the seeding site. A sample of 1022 animals (10% of the seeded population) was haphazardly selected from the total population during this process to serve as tagged tracers. They were marked by painting a spot of white nail polish on the dorsal valve and subsequently remixed with the entire seed population just prior to release.

A further four groups of 60 scallops each (including 6 marked individuals) were selected haphazardly from the seed population to serve as controls for postseeding mortality owing to factors other than predation (e.g., handling, disease). They were placed in 0.38 × 0.38 m pearl nets with a nominal (stretched) 5 mm mesh size, and suspended 2.5 m off the seabed in the study site at the time of seeding.

On November 20, 1990, 10 220 scallops (including the marked sample) were released by divers in a demarcated 10 × 4 m area by pouring the animals out of buckets from 0.5 m above the seabed. Seeding occurred at dusk (between 16:30

Table 1. Characteristics of the survey area and sampling of a seeded scallop population in the channel east of Corkum Island, Lunenburg Bay, Nova Scotia, prior to and in the year following the release of 10 220 juvenile *Placopecten magellanicus* onto the seabed.

Survey dates	Days after seeding	Survey area (m^2)	Total sample area		No. of quadrats in sample	Sample effort (h)	Sea temp. (°C)	Counts of seeded scallops	
			m^2	%				Live	Dead
20 Nov. 1990 (preseed survey)	–0.5	na	100	na	400[a]	8.3	7.0	na	na
21 Nov. 1990 (day 1)	+0.8	130	26	20	105	5.4	6.5	2147	35
26–27 Nov. 1990 (day 6)	6–7	590	89	15	356	13.1	6.5	1491	150
6–7 Dec. 1990 (day 16)	16–17	1250	179	14	716	14.5	4.5	782	129
30–31 Jan. 1991 (day 71)	71–72	520	120	23	481	11.4	1.0	555	202
20 Feb. 1991 (far-field survey)	92	3930	864	22	3456[a]	3.0	–0.5	3	nd
28 Feb. 1991 (far-field survey)	100	4640	1052	23	4208[a]	3.5	0.0	52	nd
26–27 Mar. 1991 (day 128)	128–129	1000	193	19	772	16.0	5.0	847	171
15–16 May 1991 (day 177)	177–178	1140	254	22	1017	11.9	7.0	716	370
25–26 July 1991 (day 248)	248–249	3640	470	13	1879	18.0	13.0	643	471
19 Dec. 1991 (day 394)	394	7130	571	8	2284[b]	9.3	4.5	330	nd

Note: Sample results are given in terms of the effort expended in man-hours underwater to count live and dead scallops in quadrats covering a proportional sample area of the survey area on each date. na, not applicable; nd, no data.

[a] Linear or circular transect surveys.

[b] Video transect survey, expressed in terms of 0.25-m^2 quadrats.

and 17:00 Atlantic standard time), just after slack low water. During this period, depth-averaged currents in the 0.6 m above the seabed ranged from 0.14 to 0.22 $m \cdot s^{-1}$ on 260 to 240°T. Sea temperature at the time was 6.5°C, salinity 31.2 ppt, and horizontal visibility 4.5 m.

Postseeding monitoring

The 40-m^2 release area was centered on a 50-m graduated (0.5-m interval) lead line placed parallel to the central axis of the channel (Fig. 1). The survey area on either side of the transect was quantitatively sampled by SCUBA divers using 0.5×0.5 m (0.25 m^2) quadrats on the day of seeding immediately prior to the release of the juvenile scallops, and at 16 h and 6, 16, 71, 128, 177, and 248 days after seeding (Table 1). In each survey, the numbers of live scallops, intact and broken shells of seeded scallops, and predators were counted in contiguous series of 0.25-m^2 quadrats extending perpendicularly (i.e., across channel) from both sides of randomly selected points on the central transect line. The quadrats were sampled to the edge of a predesignated survey area, or until at least three successive samples yielded zero counts of live and dead seeded scallops. Thus, the area sampled expanded as the seeded population of scallops dispersed, with marked increases in the survey area on days 6, 16, and 248 (the larger surveys took 2 days to complete, Table 1).

Far-field surveys were conducted during February (days 92 and 100, Table 1) to determine if substantial numbers of seeded scallops had moved beyond the operationally defined survey area. Semicircular search patterns were extended to 50 m radius from the northeast (towards the open bay) and southwest ends of the central transect line (Fig. 1). Another circular search was done 50 m southeast of the survey area, in Mosher Cove. Live seeded scallops only were counted in 1 m wide radii at 5-m intervals by divers holding a tape secured to a mooring.

On the last sample date (day 394) the central transect line was extended to 125 m along 215°T, and an area exceeding 7500 m^2 was surveyed with a diver-held Hi 8-mm video camera (Sony CCD-V101) in an underwater housing (Amphibico Ltd., Montréal). Moveable 50-m transect lines (graduated at 0.5-m intervals) were centered perpendicular to the central transect at randomly selected points along it. A 0.5 m wide strip of seabed was imaged along the moveable transects, using a graduated focal-distance rod to keep the camera 0.6 m above the seabed and centered on the transect line. Calibration quadrats were embedded within the video transects, and counted by a diver immediately following the passage of the camera. The video tapes were analyzed (Sony EV-5900 VCR, Sony KV-20EXR20 color monitor) in 0.5-m sections (equivalent to 0.25-m^2 quadrat samples), and the same data recorded as for quadrat surveys except that the remains of dead scallops were not counted.

The area sampled by quadrats within a given survey ranged from about 13 to 23% of the total area surveyed, while the area sampled by video in the last survey was about 8% (Table 1).

Unmarked scallops belonging to the seeded population were readily distinguished from those of the natural population on the basis of their uniform, small size and the distinct patterns of radial color bands on their dorsal valves. Seeded animals became more difficult to identify as they diverged in size because of differential growth, and as they became fouled with epibionts.

Calculated densities of living and dead seeded scallops, wild scallops, and predators were stratified according to location within the survey area. The strata were as follows: the release area (40 m^2), a predesignated 25 × 20 m area (500 m^2) centered on the release area, and an extended border area defined operationally as described above. Total abundances of each class of organisms within the entire survey area on a given date were estimated by integrating sample densities across all strata. Distance-weighted, least-squares interpolation algorithms (McLain 1972, as implemented by Wilkinson 1991) were used to plot density contours of scallop and predator abundances for each survey date. Vector analysis was used to calculate the distance and bearing from the centre point of the release area to the location of each sampled scallop (±0.25 m and ±3° accuracy) on each survey date. Radial triangulation to the outermost scallops located in each survey was used to calculate the actual area covered by the seeded population. Population dispersion rates (K_A, apparent horizontal diffusivities) were calculated from the mean square radial displacement of scallops from the centre of the release area on subsequent sampling dates, and plotted against the time since seeding (Okubo 1971).

The survival of scallops in pearl nets was monitored on each survey date until day 177. The low frequency of sampling beyond this date meant that the nets could not be adequately cleared of fouling organisms, and the observations were discontinued.

Haphazardly selected subsamples (n = 183) of the seeded and wild population were collected on the day before seeding and on day 380 (n = 230) for size-frequency analyses. Shell height was measured with vernier calipers to the nearest 0.1 mm before the animals were returned to the population.

Independent mortality assays

Rates of mortality of juvenile scallops were measured before and after seeding using arrays of animals tethered to the seabed within the survey area. Pilot studies in the laboratory demonstrated that the tethers allowed a limited escape swimming response to attack by starfish. Scallops from the same population as that seeded were attached to numbered stainless steel stakes with 15-cm lengths of 2.5-kg test monofilament fishing line glued to the dorsal valve with rapid-set epoxy glue. Groups of 25 tethered animals in the size range 7–30 mm were evenly spaced in 3 m diameter circular arrays (i.e., about 30 cm apart) by pressing the stakes into the substratum around fixed markers at three points 25 m apart along the central transect. Tethered scallops were inspected daily by divers for the first 2 days, and then at longer intervals until the number surviving decreased to about 20% of the initial number.

The causes of mortality could be inferred from the remains on the tethers. Death owing to handling stress left tissue within gaping valves in the first day; starfish predation left undamaged, empty valves; crab predation left fragments of shell; and fish predation (or other forms of mechanical separation) left a broken tether or one with glue only. To control for loss of scallops from tethers as a result of factors other than predation, eight scallops were tethered inside a 1 m diameter by 0.5 m high cage (15-mm mesh aperture) near the central array. The survival of this control group was monitored at the same time as the other arrays.

All data were analyzed using the SYSTAT and SYGRAPH computer package (Wilkinson 1991). Standard deviations are given after means, and statistical tests are reported at the p = 0.05 level of significance, unless otherwise noted. Circular statistics (Batschelet 1981) were used to test whether the distribution of the scallop displacement angles differed significantly from a random one, and from the tidal and residual current vectors for each survey independently.

Results

Preseeding conditions

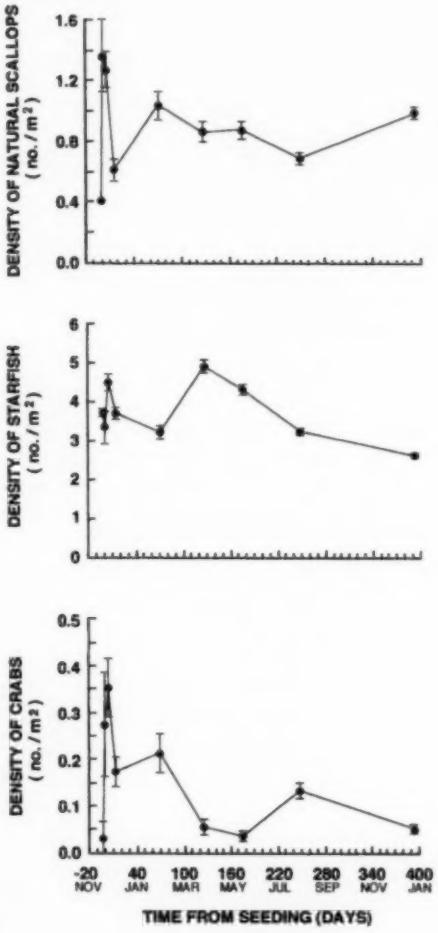
The natural population of *Placopecten magellanicus* in the survey area prior to seeding occurred at an average density of 0.40 ± 0.11 m^{-2} (Fig. 2). It had a broad size distribution (approx. 6–160 mm shell height), in which about 16% were juveniles in the size range of the seeded population (i.e., <30 mm shell height). Thus, the number of these small scallops in the 40-m^2 release area prior to seeding was negligible (~3 individuals). The preseeding density of starfish (*Asterias forbesi* and *Asterias vulgaris* in approximately equal proportions) averaged 3.71 ± 1.67 m^{-2}, and that of rock crabs (*Cancer irroratus*) averaged 0.03 ± 0.07 m^{-2} (Fig. 2). Other potential predators of juvenile scallops, such as sculpin (*Myoxocephalus* sp.), winter flounder (*Pseudopleuronectes americanus*), and moon snail (*Lunatia heros*), occurred at densities of less than 0.05 m^{-2} throughout the study.

Postseeding dynamics of the seeded scallops

Within the 40-m^2 release area, the postseeding decrease in scallop density was rapid, falling exponentially to ca. 9 m^{-2} after 16 days, increasing slightly to ca. 10 m^{-2} by day 128, and gradually declining to slightly more than 1 m^{-2} by day 394 (Fig. 3). This pattern is reflected in the densities calculated for the whole population within the entire survey area (Fig. 3), where there was no significant change from day 16 to day 177 in a mean density ranging from 2.82 to 4.63 m^{-2} in different surveys (Tukey's honestly significant difference (HSD) a posteriori multiple comparisons). The final density of seeded scallops more than 1 year after seeding (0.91 m^{-2}) approximated the density of the natural population in the same area (Figs. 2 and 3), indicating that seeding approximately doubled the abundance of scallops in the final (7130 m^2) survey area.

The total area occupied by seeded scallops increased rapidly to more than 380 m^2 over the first 16 days after seeding, when the mean displacement from the centre of the release zone reached about 10 m (Fig. 4*a*). The major axis of population movement during this period was to the south (Fig. 5). The distribution of the population appeared to contract between days 16 and 71, and the density near the centre of the survey area had increased slightly by day 128. Far-field

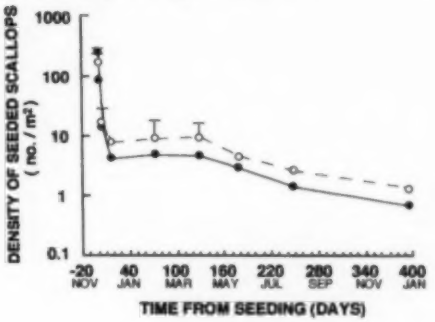
Fig. 2. Mean densities of naturally occurring scallops (*Placopecten magellanicus*), starfish (*Asterias forbesi* and *Asterias vulgaris* combined), and crabs (*Cancer irroratus*) in the survey area near Corkum Island, Lunenburg Bay, Nova Scotia, prior to and on days following seeding with juvenile scallops on 20 November 1990. Error bars represent the standard error of the sample means normalized to 1 m^2. Sample size (no. of 0.25-m^2 quadrats) ranges from 105 to 1879 (see Table 1).



surveys between these dates yielded only 3 seeded scallops in the search area northeast of the survey area (with a maximum displacement of 40 m from the centre of the release zone), and a total of 52 in the search areas to the southwest and southeast (with a maximum displacement of 50 m). These samples correspond to mean densities of less than 0.01 m^{-2} on the seabed extending out of the channel, and 0.05–0.18 m^{-2} on the seabed extending southwards and into Mosher's Cove (Fig. 1).

High initial rates of population dispersion (as measured by apparent horizontal diffusivity) during the 16 days after seeding dropped close to zero between days 16 and 71 (Fig. 4*b*), reflecting a relatively static distribution of the scallop population during this period (Fig. 5). The rate of

Fig. 3. Mean (+SE) density of juvenile scallops (*Placopecten magellanicus*) during 13 months following release on the seabed near Corkum Island within the 40-m^2 release area (open circles), and in the entire survey area as it expanded from 40 to 7130 m^2 (solid circles). Where invisible, the SE falls within the symbol. Sample sizes given in Table 1.



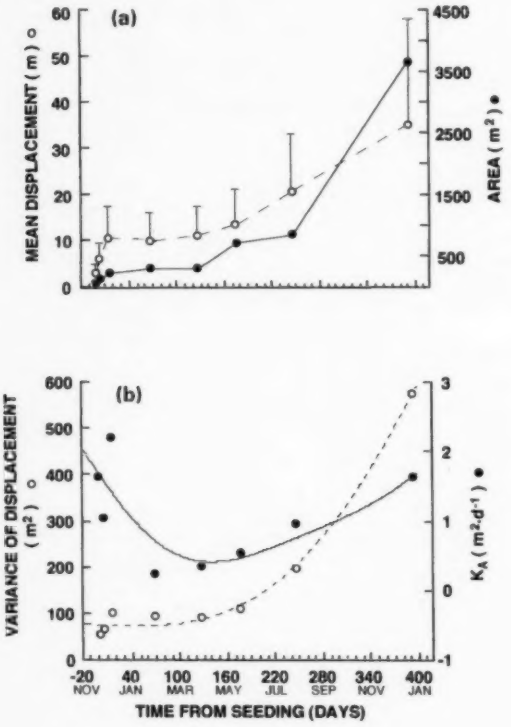
dispersion increased steadily over the next 5 months (spring–summer), causing a doubling in the mean displacement distance from 10 to about 20 m (Fig. 4). The final dispersion rate over the last 5 months of the study reached a high value similar to that immediately after seeding. The population spread over an area in excess of about 3500 m^2, with a mean individual scallop displacement of about 35 m (Fig. 4*a*). The furthest recorded displacement of a marked individual was 93 m on 140°T from the centre of the release zone.

The fitted exponential diffusion curve (Fig. 4*b*, $r^2 = 0.925$, $P < 0.001$, $n = 8$) corresponded to an average population dispersion rate (K_A) of 0.98 $m^2 \cdot day^{-1}$ over the 394-day period. No significant linear relationship existed between the dispersion of the seeded scallop population and its length scale (radially symmetrical area, $F_{[1,8]} = 2.11$, $P > 0.1$), demonstrating that diffusion was non-Fickian (Okubo 1971). K_A was poorly described by a quadratic regression (Fig. 4*b*), but was significantly correlated with mean ambient water temperature between survey dates over the course of the experiment ($r = 0.757$, $P < 0.01$, $n = 8$). Dispersion rates were low in winter and spring, when average sea temperatures were below 5°C.

The spatial distribution of the seeded population during the first 8 months showed a consistent orientation towards the southern and eastern portions of the survey area (Fig. 5). The distribution of scallop displacement vectors differed significantly from random in all surveys (Rayleigh's test, $P < 0.01$), and the mean displacement angles differed significantly between the eight surveys (Watson–William's test, $F_{[7,750]} = 17.81$, $P < 0.001$). During the last 5 months of the study a substantial westerly expansion of the distribution towards the centre of the channel occurred. The pattern of scallop distribution did not correspond well to the tidally dominated pattern of water movement immediately above the seabed at the release site (Figs. 1 and 5). The mean angle of net scallop movement from the release point varied from 137 to 176°T between surveys over the duration of the study. These mean angles differed from the main tidal current axes (ebb = 352°, flood = 233°; Rayleigh's tests, $P < 0.001$) and the calculated Eulerian residual current (330°T).

The abundance of dead scallops within the survey areas

Fig. 4. Dispersion characteristics of a population of juvenile scallops (*Placopecten magellanicus*) during 13 months following release in the centre of the survey area near Corkum Island, Nova Scotia. (*a*) The mean displacement distance of seeded scallops from the centre of the release area (open circles) and the total area in which live scallops were found (solid circles) are shown for each survey date. Errors bars represent one half of the standard error of the mean. (*b*) The variance of the individual scallop displacements (open circles) and the apparent horizontal diffusivity of the seeded scallop population (solid circles) are plotted as a function of time since release. Exponential and quadratic curves are fitted (maximum likelihood) to the data. Sample sizes range from 2147 to 330 scallops.



showed no consistent pattern during the study. The spatially averaged density of dead animals varied significantly (ANOVA, $F_{[6,5319]} = 8.373$, $P < 0.001$) over the 248-day period in which it was measured, reaching a low of 0.72 m^{-2} on day 16, peaking at 1.69 m^{-2} 6 days after seeding, and averaging 1.00 m^{-2} after 248 days. The estimated total numbers of dead scallops at each survey ranged from 106 on day 1 to 3640 on day 248 (Fig. 6). Their spatial distribution showed a consistent pattern of constant numbers and high density (4–8 m^{-2}) in the release area from day 6 to day 177, and low densities (<1 m^{-2}) in the surrounding areas. The high-density areas did not overlap those of the live population (which progressively extended south-southeast, Fig. 5) beyond day 1. The expansion of the total area in which the remains of dead scallops were found after day 6 did show a similar pattern to the outer boundary of the distribution of the live animals, with an extensive, diffuse distribution by day 248. The abundances of dead and live seeded scallops within quadrats were significantly correlated (Pearson product moment correlation) on days 1, 71, 128, and 177, with correlation coefficients ranging from 0.105 to 0.264. The abundances of empty intact valves and broken valves of dead scallops was correlated on all survey dates, with Pearson multiple correlation coefficients ranging from 0.13 to 0.46 ($n = 272–1017$).

The estimated size of the surviving scallop population ranged from just under 10 000 individuals 16 h after seeding, to less than 2500 individuals on day 71 (Fig. 5). Survivorship dropped sharply over the first 2 weeks following seeding (Fig. 7), corresponding to losses from the population of about 450 and 350 animals/day during the first and second weeks, respectively. Subsequent estimates of population size fluctuated between about 4900 and 3200 individuals (just under one half to one third of the seeded population) as the survey area was expanded (Figs. 5 and 7). The low precision of these estimates based on approximate area measures (described in Methods) and density measures with high sample variances (Fig. 3) prevented the detection of appreciable differences in the size of the seeded population beyond day 128, giving similar magnitudes at periods of 16, 128, and 248 days after seeding.

Predator populations

The densities of starfish and crabs were highly variable within the survey areas and did not exhibit clear patterns of temporal variability over the course of the study (Fig. 2). The time-weighted mean starfish density was 3.40 ± 1.33 m^{-2} ($n = 9$). It changed by less than a factor of two throughout the year, being significantly elevated in the spring and early summer surveys (ANOVA: Tukey's HSD tests, $P < 0.05$). Starfish density did not change significantly on the day following seeding, but was significantly greater by day 6 (Fig. 2).

The spatial distribution of starfish (Fig. 8) did not demonstrate aggregation of these predators within the high-density areas of either live or dead scallops (Figs. 5 and 6). Metres-scale patches of high starfish density were observed on days 6, 128, 177, and 248 (Fig. 8). These were located on the boundaries of the survey grid, including the south-southeast quadrant on day 177 (the area towards which the centroid of scallop distribution moved following seeding). The high variance among 0.25-m^2 quadrat samples within the survey area (Fig. 2) demonstrates the small-scale patchiness of starfish distribution at this site.

The time-weighted mean crab density during the study period was 0.14 m^{-2} ($n = 9$). It varied by more than an order of magnitude during the course of the study, although no seasonal pattern of crab abundance is apparent (Fig. 2). The high variance among 0.25-m^2 quadrat samples within the survey area (mean CV = 1.82, $n = 9$) demonstrates the extreme patchiness of crab distribution at this site. Crab density did increase significantly over the 16 h immediately following the release of scallops (ANOVA: Tukey's HSD tests, $P < 0.05$), then decreased after day 6 to consistently low levels (and reduced spatial variability) for the last 8 months of the study (Fig. 2).

None of the other species of potential benthic predators (i.e., moon snails, lobsters, sculpins) increased significantly during the period following scallop seeding. At no time in over 100 h of diving on the site were any itinerant schools of fish

Fig. 5. Spatial distribution of a population of 10 220 juvenile scallops (*Placopecten magellanicus*) during 13 months following release on the seabed in a channel near Corkum Island, Nova Scotia. The smallest box in each plot represents the 40-m^2 release area. Larger boxes represent the expanded survey area (note change in scale of plot for day 394). The orientation of the along-channel axis is towards 25° (north-northeast), and the mean tidal current vectors are shown in the first contour plot. The total number of scallops estimated from sampling within the survey area (and the percentage this represents of the original seeded population) is given above each plot (survey details in Table 1).

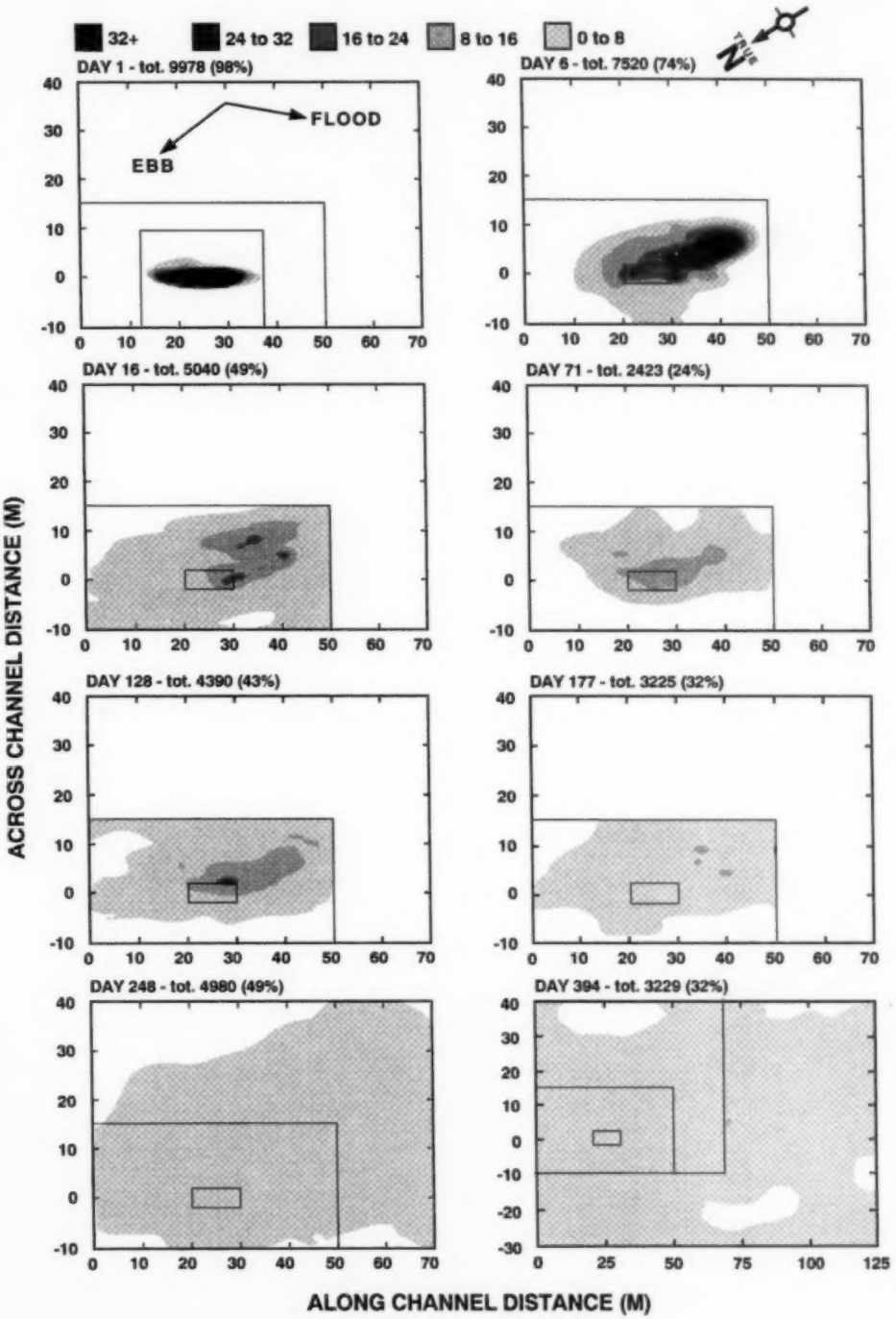
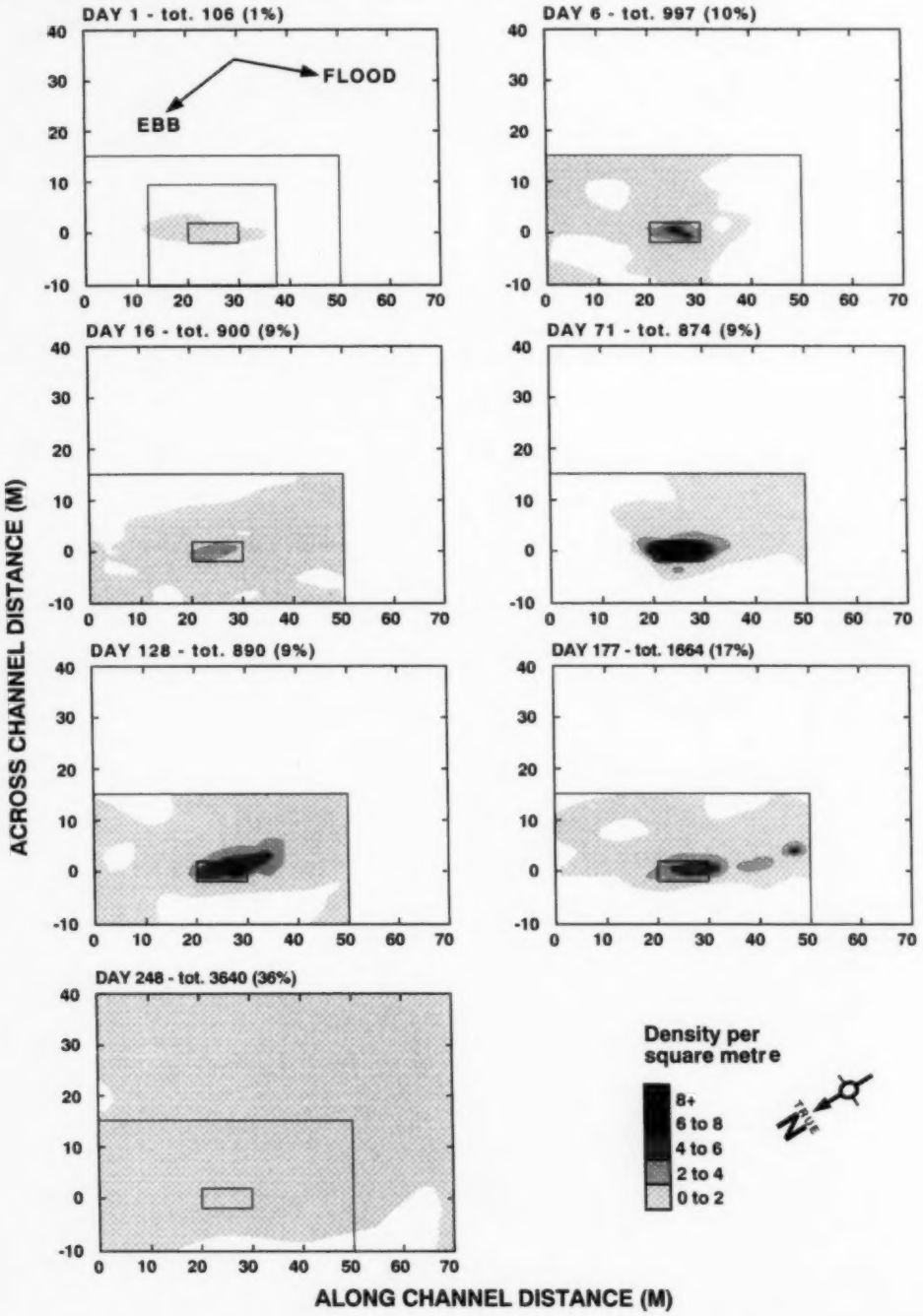

32+
24 to 32
16 to 24
8 to 16
0 to 8
DAY 1 - tot. 9978 (98%)
FLOOD
EBB
DAY 6 - tot. 7520 (74%)
DAY 16 - tot. 5040 (49%)
DAY 71 - tot. 2423 (24%)
DAY 128 - tot. 4390 (43%)
DAY 177 - tot. 3225 (32%)
DAY 248 - tot. 4980 (49%)
DAY 394 - tot. 3229 (32%)
ACROSS CHANNEL DISTANCE (M)
ALONG CHANNEL DISTANCE (M)

Fig. 6. Spatial distribution of the dead remains of juvenile scallops (*Placopecten magellanicus*) during 8 months following the seeding of 10 220 individuals on the seabed in a channel near Corkum Island. Survey details as in Fig. 5.



with the potential to prey on scallops (e.g., cod, pollock, flounder) observed. The densities of crabs and rarer predators were too low for useful contour plotting.

The abundances of starfish, crabs, or any other predator species within quadrats were not significantly correlated with the abundance of live or dead scallops, or with each other on

any of the survey dates (Pearson multiple correlation, $P > 0.05$).

Scallop mortality

Most of the mortality in the four tethering experiments was attributable to starfish predation, as 60–80% of the remains were clean, undamaged valves (Fig. 9). Only about 1% of intact valves had flesh within them on the first day after release, indicating low nonpredatory mortality. Between 0.5 and 8% of the remains were broken valve fragments indicative of crab predation. The remainder of the losses left no remains, and were attributed to removal of scallops by crabs, fish, or rarer predators, because detachment of scallops from tethers in the cage control was <3%. The ratios of broken to intact valve remains in the seeded population of scallops ranged from 0.02 to 0.11 on different survey dates, which were much lower than the ratios obtained from the tethering experiments.

Arrays of tethered scallops within the survey areas suffered high rates of mortality prior to and in the 2 weeks following seeding (Fig. 9), there being no significant difference in the initial slopes of the survivorship curves between these two periods ($t = 0.431$, $P > 0.1$). The estimated time to 80% mortality in these experiments was between 10 and 20 days, and the proportion of the mortality resulting from crab predation increased following seeding. Mortality during the winter and early spring (70 to 157 days after seeding) was significantly lower (ANOVA, Tukey's test, $P < 0.005$), with a time to 80% mortality of over 60 days. By late spring mortality had again increased such that the survivorship curve overlapped those of the autumn experiments, with a time to 80% mortality of about 15 days. The rate of predation loss from tethering arrays during the first 10 days of each experiment was correlated with the average sea temperature during that period ($r = 0.960$, $P < 0.05$, $n = 4$).

Scallop mortality rates derived from the tethering experiments were markedly higher than those derived from change in the estimated size of the seeded population. The initial slope of the population survivorship curve (Fig. 7) corresponded to a loss rate of about 3.2%/day over the first 16 days after seeding, which was less than half that of 7.8%/day measured in the concurrent tethering experiment (Fig. 9). More significantly, the survivorship curve approached an asymptote between 24 and 49% surviving after about 3 months, while the extended tethering curves continued to decline below 20%. These contrasts are conservative, because the population survivorship curve does not include animals that had moved outside the survey areas.

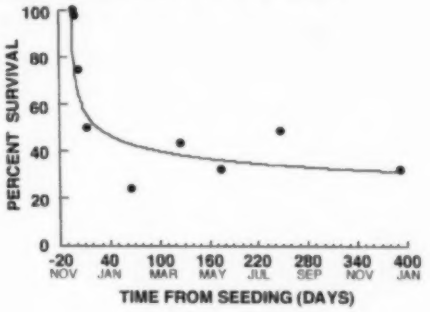
The total mortality of seeded scallops estimated from sampled densities of remains was about 1% on day 1, reached about 10% on day 6 and remained at this level until day 128, and then increased to reach 36% of the seeded population by day 248 (Fig. 6). These mortalities ranged from 10% (day 71) to 70% (day 248) of the losses estimated from the densities of live animals (Figs. 5 and 7) and were much lower than the independent mortality rate measurements (Fig. 9).

There was no mortality of scallops kept in pearl nets at the study site during the first 177 days of the experiment.

Scallop growth

The size-frequency distributions of the seeded scallop population at the time of seeding and on day 394 approximated normal distributions with nonoverlapping tails (Fig. 10). The hand-measured sample taken on day 380 and the video sample from day 394 yielded similar means ($t = 0.312$, $P > 0.5$) and variance ($F = 1.03$, $P_{[\text{two-tailed}]} > 0.2$) distribution statistics, so the larger video sample was used to characterize the final population. Over the course of the experiment the range of shell heights diverged from an initial spread of 4–26 mm (22 mm) to a final range of 36–66 mm (30 mm). The means did not diverge markedly from the modes of samples in either the initial or final surveys, which allowed prediction of the average shell growth of individuals within the population over the duration of the study. The size increment of 34.9 mm over the 13-month growth period corresponds to an average growth rate of 0.09 mm·day^{-1}.

Fig. 7. Temporal variation in the survivorship of a population of juvenile scallops (*Placopecten magellanicus*) released on the seabed in a channel near Corkum Island, Nova Scotia, on 20 November, 1995. The least-squares negative exponential curve has a slope of –0.0014 ($r^2 = 0.160$, $n = 9$).



Discussion

Interpretation of the trajectory of the seeded population

Accurate description of the trajectory of the seeded scallop population depends on the ability of the sampling scheme to estimate change in abundance through space and time. Rates of survivorship can only be inferred if the entire population is sampled representatively. Sampling to the edges of the population's distribution is required to avoid scoring emigration as mortality, although it is not essential for good estimates of rates of horizontal dispersion. Our sampling probably failed to encompass the outermost distribution of the population beyond day 6, thereby confounding the effects of dispersion and mortality in estimating true population size. The relative importance of these processes in determining observed changes in scallop density cannot be unequivocally resolved from the abundance data alone. The problem is common to all seeding experiments in natural habitats (e.g., Morgan et al. 1980; Tegner and Butler 1985; Bull 1989; Dao et al. 1994), and its resolution requires advances in techniques for surveying low densities of animals over extensive areas of seabed.

The four surveys on days 16 to 177 (inclusive) underestimated the actual population by that proportion which had dispersed beyond the limits of the 50 × 25 m survey area (Fig. 5). The sample on day 71 was biased by the effects of cold water, strong currents, and poor visibility on divers'

Fig. 8. Spatial distribution of starfish (*Asterias forbesi* and *Asterias vulgaris*) during 13 months following release of a population of juvenile scallops on the seabed in a channel near Corkum Island, Nova Scotia. The total number of animals estimated from sampling within the survey area (and the calculated mean density throughout that area) is given above each plot. Other survey details as for Fig. 5.

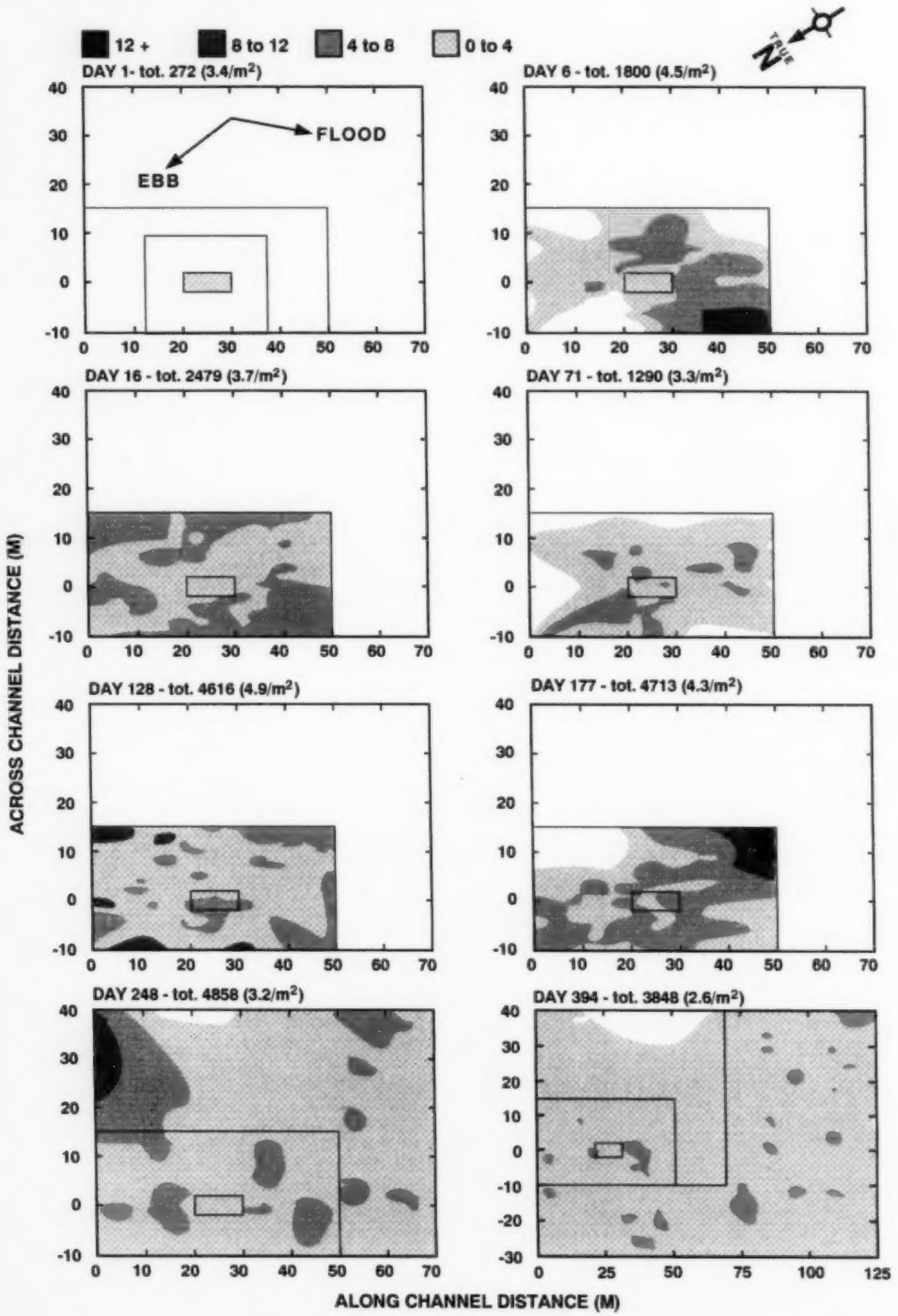

12 +
8 to 12
4 to 8
0 to 4
TRUE N
DAY 1- tot. 272 (3.4/m²)
FLOOD
EBB
DAY 6 - tot. 1800 (4.5/m²)
DAY 16 - tot. 2479 (3.7/m²)
DAY 71 - tot. 1290 (3.3/m²)
DAY 128 - tot. 4616 (4.9/m²)
DAY 177 - tot. 4713 (4.3/m²)
DAY 248 - tot. 4858 (3.2/m²)
DAY 394 - tot. 3848 (2.6/m²)
ACROSS CHANNEL DISTANCE (M)
ALONG CHANNEL DISTANCE (M)

Fig. 9. Survivorship (*a*) and evidence of mortality (*b*) of groups of juvenile scallops (*Placopecten magellanicus*) tethered in the seabed survey area of a channel near Corkum Island, Nova Scotia, prior to and during seeding on November 20, 1995. Cluckers are clean, undamaged, but empty valves. Experiment 1, preseeding period, 23 Oct. – 15 Nov. 1990; experiment 2, postseeding period, 26 Nov. – 6 Dec. 1990; experiment 3, postseeding period, 29 Jan. – 26 Apr. 1991; experiment 4, postseeding period 9 May – 18 June 1991. Error bars represent the standard error of the sample means. Samples consisted of three replicate arrays with 25 tethered scallops in each.

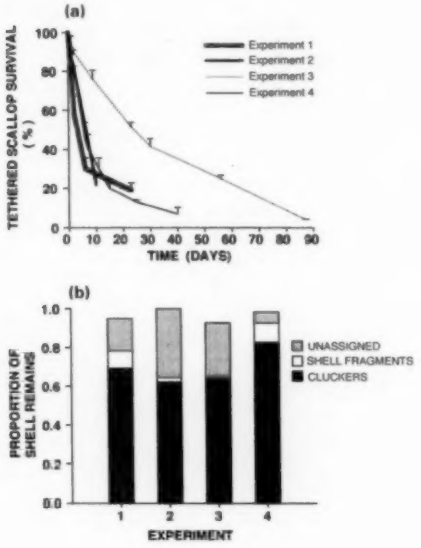
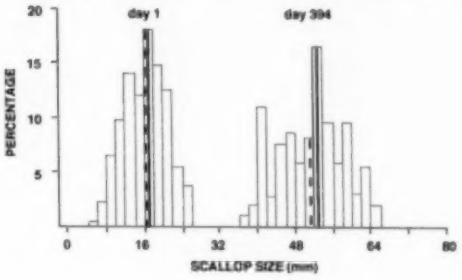


Fig. 10. Frequency distribution of shell heights of individuals in a population of juvenile scallops (*Placopecten magellanicus*) released in a channel near Corkum Island, Lunenburg Bay, Nova Scotia, measured on 20 Nov. 1990 (day 1) and on 19 Dec. 1990 (day 394). The mean (solid lines) and median (broken lines) sizes are shown. Sample sizes are 183 and 393 individuals, respectively. The histogram interval is 2 mm.



ability to discern the seeded scallops. The count in the next survey (day 128) suggests that this poorest sample underestimated the population by about 20%, while the few scallops discovered in subsequent far-field surveys demonstrate that only a small proportion (<5%) lay beyond the survey area at that time. The large expansion of the survey grid on day 248 yielded the highest proportion of the population sampled after day 16. High labour costs incurred in this survey (Table 1) precluded further expansion of quadrat sampling, justifying the switch to video transects on day 394. This last sample recorded the continued dispersion of the seeded scallops. It suffered from the increased difficulty of distinguishing between natural and seeded animals in video images taken more than a year after release. Subsequent calibration of video scallop sampling yielded an underestimation factor of 1.274, resulting in a corrected final population estimate of 4114 scallops (40% of the number seeded) in the survey area on day 394. This final number underestimates the actual survivors by the unknown, but probably small, number of scallops that had dispersed beyond the survey area.

Given the necessity of matching the sampling area to the distribution of the scallop population, and the relative costs and benefits of underwater survey methods, we conclude that the video transect method is superior to the randomized quadrat method after the first few weeks postseeding. Video census techniques have been employed successfully in extensive surveys of scallop stocks elsewhere (e.g., Bull 1989; Thouzeau et al. 1991).

A realistic interpretation of the trajectory of the seeded scallop population and the processes controlling it is possible within the constraints of the sampling regime discussed above. We identify four phases. The initial response of juvenile scallops released onto the seabed at over 200 times the natural density was to disperse rapidly (Figs. 4 and 5). This was accomplished by jumps (i.e., single contractions of the valves) and brief swims that moved scallops a few centimetres, such that they were not touching one another. They then secured themselves to small rocks and shells with byssal threads, but could swim again quickly if necessary (as shown in other studies, e.g., Caddy 1972; Parsons et al. 1992).

Little mortality accompanied the rapid dispersion of the seeded scallops during the initial phase, as documented by the numbers of live and dead animals censused 16 h after release (Figs. 5 and 6). We attribute the 2% loss in this period primarily to mortality induced by handling stress prior to and during release, rather than to predation. Losses of 12–15% of scallops during the initial 16–30 h of tethering experiments initiated around seeding (Fig. 9) also included a small component (<5%) of handling mortality. Seeding scallops at high density may have inhibited predation on healthy animals by swamping the local predator population with injured prey.

There is some evidence that predators aggregated in the area occupied by the seeded scallops in the first few hours after release (Figs. 2 and 8). Crabs could have converged rapidly on the seeded population, as suggested by the sharp increase in density. The sampling regime was inadequate to detect a statistically significant increase in the abundance of starfish.

The continued aggregation of the scallop population within the survey area as the centre of density moved south-southeast over the next 5 days (day 6, Fig. 5) gives us confidence that the estimated 24% loss during this period was due primarily to mortality. Predation by both crabs and starfish was observed by divers; however, although the average starfish density increased significantly (32%) during this period the starfish were concentrated in the western corner of the survey area, about

10 m away from the bulk of the seeded scallops. Despite an 80% decrease in mean scallop density, encounter rates with predators (the major determinant of juvenile scallop predation rates, Barbeau and Scheibling 1994*a*) most likely increased during this phase as the seeded scallops dispersed and predator densities increased.

We calculate that only about 150 starfish and two crabs were within the 40-m^2 release area at the time of scallop seeding. Total abundances of the two predators within the spatial extent of the scallop population had increased to ca. 2000 and 150, respectively, by day 6. Laboratory measures of feeding rates of these predators on similar-sized scallops at similar densities (Barbeau and Scheibling 1994*a*) indicated that they could have consumed about 1600 and 2300 scallops, respectively, over the period. These extrapolations from laboratory measures are of the same order as mortality derived from the scallop survival data (Fig. 7), and suggest a disproportionate effect of crab predation relative to their abundance within the distribution of the seeded scallop population (a suggestion supported by subsequent tethering experiments, Barbeau et al. 1994).

Scavenging and decomposition of soft tissues made handling mortalities during the first few days indistinguishable from mortalities resulting from starfish predation. We cannot exclude the possibility that a large proportion of the loss during the first 6 days (perhaps as much as 15% of the seeded population) was due to handling mortality. The distribution of dead scallop remains on day 6 was discontinuous and more diffuse than that of the live population, extending to the northern limits of the survey area from a higher density centroid in the release area (Fig. 6). The remains were mostly joined or single intact valves, rather than valve fragments. This pattern is consistent with rapid mortality in the release area and passive advection of shells in strong ebb tide currents. The fact that the number of dead scallops counted was a fraction of the loss estimated from counts of survivors (only about 36% of the seeded population at day 6) may reflect rapid dispersion from the survey area, or burial of remains in resuspended sediments. The survey on day 71 also revealed a high density of dead scallop remains (mean > 8 m^{-2}) centered in the release area, which may have been uncovered by spring tide currents prior to this survey.

Mortalities directly caused by handling should have occurred by day 6, and we attribute the continued rapid loss of animals over the next 10 days primarily to predation as the population continued to disperse rapidly (Figs. 4 and 5). Reduced condition during recovery from handling stress may have contributed indirectly to mortality in this period by making some scallops less able to resist attack by predators. The rate of mortality measured in the tethering experiments also remained high during this second phase (Fig. 9). Independent experiments have shown that tethered scallops are captured by starfish at almost 17 times the rate as unrestrained scallops, while tethering has no significant effect on the rate of crab predation (Barbeau and Scheibling 1994*b*). After correcting the mortality rates derived from the tethering experiments for this bias, we calculate a loss of 4286 scallops (41% of the seeded population) after 16 days, which is almost five times the number of dead scallops observed (Fig. 6), but is similar to the 51% mortality inferred from counts of survivors (Fig. 5).

Swimming escapes from predators are likely to have accounted for the substantial displacement of scallops during this period, because attack by predators is the primary trigger for scallop swimming (Brand 1991). By day 16 the edges of the population extended beyond the 50 × 25 m survey area (Fig. 5), although the resultant overestimate of mortality was a small proportion of the population at this time, as indicated by subsequent far-field surveys.

A third phase of the seeded population's trajectory extended from about 2 weeks to 6 months after seeding, during the winter and spring. It was characterized by reduced scallop movement and mortality. The apparent reduction in the size and extent of the population by day 71 is an artifact of inadequate sampling on this date. Dispersion rates near zero (Fig. 4) and an increase in scallop density near the centroid of distribution between this survey and the subsequent one on day 128 suggest some reaggregation of scallops during midwinter, but we can offer no convincing explanation for this pattern. The frequency and duration of scallop swimming and the activity of predators are known to decrease markedly at the low water temperatures (Manuel and Dadswell 1991; Barbeau and Scheibling 1994*c*; Kleinman et al. 1995*b*). Survivorship in the tethering experiment during this period also showed markedly reduced predation rates within the survey area (Fig. 9).

A fourth phase of dispersion had begun by midspring (day 177), when the scallops that were previously concentrated southeast of the release area had dispersed (Fig. 5). The population's accelerated dispersal can be attributed to a resumption of scallop swimming, reaching a mean displacement of over 20 m by day 248 (Fig. 4). The extended quadrat survey on that date gave an estimate of the seeded population similar to that of a survey of one third of the area on day 16, implying no significant mortality during the intervening period. Mortality undoubtedly occurred over the winter, and the similarity of the estimates is an indication of the numbers of animals that were missed in the more restricted surveys on days 71 and 177. Over 1 year after seeding (day 394), the scallop population had dispersed well beyond the boundaries of the largest survey area (Fig. 5), making the continued estimation of population size impossible.

The uncertainty of detecting scallop remains and the likelihood of recounting them in subsequent surveys mean that estimates of mortality based on censuses of dead scallops are of dubious value. Taken as a single measure of cumulative mortality, the value of 3640 dead scallops calculated from the survey on day 248 provides a minimum estimate of predation mortality of about 36% of the seeded population by that date, which represents two thirds of the total estimated losses from the population on the basis of counts of survivors (Figs. 5 and 7).

In summary, four phases were observed in the trajectory of the seeded scallop population during the 13 months following its release in late autumn of 1990. (*i*) The first was a sorting phase for the first few hours during which the highly aggregated scallops separated themselves and attached to the substratum. Handling injuries caused most mortality during this period and dispersion was limited. (*ii*) The second was a mortality and dispersion phase over the first few days to weeks when predation was severe (especially on animals weakened by handling), and frequent long swims (in response to predator attack) produced large individual displacements and rapid

horizontal diffusion of the population. (*iii*) The third, stable phase extended for several months through the winter when mortality caused by predation was low and dispersion slowed (or even stopped). (*iv*) The fourth phase was a dispersion phase extending through the summer and autumn when displacement increased to produce a uniform, low density of scallops that experienced little further mortality.

Comparisons with other seeding trials

The losses caused by dispersion and mortality reported here are within the range of those measured in seeding trials of other scallop species seeded elsewhere. Cumulative losses of 50–99% after grow-out periods of up to 2 years have been reported, with most of the losses occurring in the first few weeks after release, and the heaviest mortality in the smaller (<30 mm) size-classes (Yamamoto 1960; Morgan et al. 1980; Ventilla 1982; Volkov et al. 1983; Dao et al. 1994; Buestel et al. 1989; Bull 1989). None of these studies unequivocally partitioned losses between predation and dispersion, but the best estimate suggested that the two processes accounted for approximately equal proportions of 40 000 *Argopecten irradians* seeded in a shallow New York bay at densities and sizes similar to those in this study (Morgan et al. 1980).

Trial seeding of *Placopecten magellanicus* onto sand and gravel bottoms at 12 m depth near the Magdelan Islands in the Gulf of St. Lawrence (Picard 1992) provides the most comparable data to our study. Separate releases of about 57 000 and 33 000 scallops between 23 and 53 mm shell height into an area of 8100 m^2 in August 1990 and June 1991 resulted in survivorships of only 6.7 and 1%, respectively, after 20 days. Live scallop density within the seeded area had decreased by 57% on the day following the second seeding, and reached zero by day 39. The mortality component was estimated to be at least 50%, with the remaining losses attributed to rapid dispersion across an unsuitable (sand) substratum (Picard 1992). These high rates of loss during the early postseeding phases greatly exceed those measured in the present study, despite the relatively low seeding densities of 4–7 m^{-2} used in the Magdelan Islands experiments. The contrast between the two studies emphasizes the importance of careful handling of the scallops prior to seeding, and their release onto suitable substrata (i.e., those known to support natural scallop populations).

Controls on dispersion patterns and rates

Little evidence exists for directed movement (migration) by scallops, and most studies (including those of seeded juveniles discussed above) conclude that any directionality of scallop dispersion is controlled by near-bed water movement (e.g., Baird 1966; Moore and Marshall 1967; Melvin et al. 1985; Minchin 1992). Hydrodynamic measurement regimes are rarely appropriately matched with the time and space scales of the scallop population dispersion in the field (Orensanz et al. 1991; but see Carsen et al. 1995). In this study, the clear directionality of population movement was not related to that of the near-bed Eulerian mean currents measured at the release site during January 1991 (Figs. 1 and 5). Calculated Eulerian residual currents during this period did not match the predominant direction of scallop displacement either. Water currents during the first weeks following the release may have differed from those measured in January, but subsequent current records from the site and the predictions of a fine-scale numerical model (Sturley 1992; Sturley et al. 1993) support the conclusion that the tidal vectors shown in Figs. 1 and 5 accurately depict the dominant near-bed currents throughout the course of the experiment.

One explanation for the observed pattern of dispersion is that the scallops swam more often in rare southeasterly currents during slack tides or the hourly flow oscillations (which can briefly halt flows in the channel, Sturley and Leal 1991), and were thus progressively advected in that direction. Alternatively, the scallops may have swum southeast when currents were weak. In either case, appropriate triggers and orientation for swimming based on environmental clues must be postulated. Scallops are known to swim more often when placed on unsuitable substrata (Brand 1991), but the suitability of the cobble and silt seabed in the release area is demonstrated by the stable, natural scallop population it supports, and the substratum varies little throughout the survey area. Shallow Mosher Cove (Fig.1), towards which the majority of seeded scallops moved, has a sandy-mud sediment unsuitable for byssal attachment by juveniles, and few were found there. It is possible that some distinctive chemical or physical signature of the water flushing the marsh and seagrass-edged cove provided a directional cue to orient scallop swimming.

The average net displacement of 34 m over 13 months (Fig. 4) is small in absolute terms, when compared with the net displacements of a few kilometres over several years measured for tagged scallops in offshore habitats (Baird 1966; Posgay 1981; Melvin et al. 1985). It is, however, of the same order (i.e., tens of metres) as measurements made of this and other species in shallow coastal environments (e.g., Baird 1954; Moore and Marshall 1967; Chapman et al. 1979; Winter and Hamilton 1985; Minchin 1992; Parsons et al. 1992).

The horizontal diffusion diagram for the seeded scallop population is nonlinear (Fig. 4*b*), and the calculated dispersion rates are orders of magnitude slower than those obtained from measurements of conservative tracers in coastal waters (Okubo 1971). Scallop dispersion is not a simple diffusive process. Large seasonal variations in the rate of dispersion during the course of the experiment did not match the relatively minor changes in the hydrodynamic regime at these time scales (Sturley 1992; Emerson et al. 1994). They were, however, well related to variation in sea temperature, with lowest dispersion rates at times when sea temperature was less than 5°C (Table 1, Fig. 4). Temperature influences the frequency and duration of swimming in scallops primarily through its effect on the physiology of the abductor muscle (Bricelj and Shumway 1991; Kleinman et al. 1995*b*). Reduced predator activity at low temperatures (McKenzie 1969; Barbeau and Scheibling 1994*c*) would also serve to reduce the frequency of escape swimming in response to attack. We conclude that these factors were responsible for the long period of slow dispersion during the winter and spring.

Responses of predator populations

Scallops are attractive prey. Morgan et al. (1980) identified 20 potential predators of *Argopecten irradians* in their seeding areas, and concluded that decapod crustaceans caused the greatest mortalities. Starfish caused most predation mortality in seeded populations of *Patinopecten yessoensis* (Ventilla 1982; Volkov et al. 1983), while predation by both crabs and

starfish was identified as the major causes of mortality in a near-shore population of juvenile *Pecten maximus* by Minchin (1992). Small scallops of the sizes typically released in bottom culture are particularly vulnerable to predation (Yamamoto 1960; Elner and Jamieson 1979; Barbeau and Scheibling 1994*a*), and most successful commercial operations include predator eradication prior to seeding (Ito 1991). In this study, starfish and crabs can be unequivocally identified as the major agents of mortality on the basis of the tethering experiments. The apparent dominance of scallop mortality by starfish predation in tethering experiments (Fig. 9) is offset by bias of the method towards starfish predation (Barbeau and Scheibling 1994*b*). Interpreting the tethering results in light of this bias suggests that the importance of crab predation was similar to or greater than that of starfish predation as an agent of seeded scallop mortality.

Aggregation of starfish within days to weeks following scallop seeding has been shown to increase local densities of these predators by an order of magnitude in some studies (e.g., Volkov et al. 1983; Picard 1992). In other habitats and locations (including this study) there was little or no aggregative response, despite the presence of starfish in the area prior to seeding (Morgan et al. 1980; Figs. 2 and 8). These differences may reflect the relative abundance of alternative, preferred prey items in the seeding area (e.g., mussels), or a scallop dispersion rate that exceeds the starfish aggregation rate. The latter explanation is supported by the mismatched distributions of seeded scallops and starfish (Figs. 5 and 8).

Crabs and other decapod predators, such as lobster, may also respond to increased prey density by aggregating (e.g., Eggleston 1990; Picard 1992), as their high motility allows a rapid response. Our data indicate that the density of crabs did increase in the survey area during the 6 days immediately following scallop seeding (Fig. 2), but the absolute number of crabs involved is so small that we question the meaning of the result. The well-documented functional response exhibited by these efficient predators (e.g., Eggleston 1990; Sponaugle and Lawton 1990; Barbeau et al. 1994) may also have contributed to the rapid increases in scallop mortality over the first 2 weeks, in the absence of large or sustained increases in predator density during this period.

The processes of predation and dispersion are strongly interactive because attack by predators is the primary cause of scallop swimming (Caddy 1968; Stephens and Boyle 1978; Peterson et al. 1982; Winter and Hamilton 1985), and predator behavior is often dependent on prey density (Boulding and Hay 1984; Caddy 1989; Eggleston 1990; Mansour and Lipcius 1991). The frequency of swimming may also be density dependent; at high scallop densities movement by one scallop often triggers an escape response in others (Brand 1991). In our study, the rate of dispersion and predation (as inferred from mortality estimates) covaried over the first 6 months of the experiment, when most losses from the seeded population occurred. Rates of both processes decreased in winter and spring as sea temperature fell. Losses to predation and dispersion appear to have become decoupled during the last half of the study period as the population continued to spread with low mortality during the summer and autumn. An increased frequency of unsuccessful attacks by predators during this period, as the scallops grew to larger sizes that were more resistant to predation and became more efficient swimmers, explains the observed pattern and is supported by laboratory and field experiments (Barbeau and Scheibling 1994*c*, 1994*b*; Barbeau et al. 1994).

Implications for aquaculture

Approximately 40% of the scallops seeded in this experiment were alive within ca. 100 m of the release site after more than 1 year on an unconditioned natural seabed. The average individual growth rate of 0.09 mm·day^{-1} during that period is similar to that of *Placopecten magellanicus* juveniles in natural populations (Dadswell et al. 1988; Wildish et al. 1988; Naidu 1991) and to that measured in suspended culture of the species in Lunenburg Bay (Emerson et al. 1994; Kleinman et al. 1995*a*) and elsewhere (Parsons and Dadswell 1992; Naidu 1991). The growth and survivorship compare favourably with those from commercial bottom culture of other species in Japan (Ito 1991) and Europe (Ansell et al. 1991) and are markedly better than those obtained from trial seedings of the same and other scallop species elsewhere (e.g., Morgan et al. 1980; Volkov et al. 1983; Picard 1992). The final density of harvestable seeded scallops was approximately equal to the final density of the natural population, but more than twice the density of natural scallops in the channel at the time of seeding (i.e., the abundance of natural scallops increased during the course of the experiment). Thus, a two- to five-fold increase in total scallop density (up to about 2 m^{-2}) was achieved, while maintaining survivorship at about 40% and growth rates within the normal range for the species.

The major differences between this and other less successful bottom seedings of scallops are (*i*) low preseeding handling stress, (*ii*) choice of suitable substratum, (*iii*) lack of large numerical aggregative responses by predator populations, and (*iv*) a long period of reduced predation afforded by an autumn seeding. These factors served to ameliorate the initial mortality rate and allowed substantial numbers of survivors to become established in low risk conditions through movement and growth. We do not predict substantial increases in mortality over the next year of grow out, at which time the surviving scallops will have reached harvestable size. The timing and density of the seeding relative to seasonal patterns of physical variables and predator behavior undoubtedly influenced the success of the trial. Larger seeding experiments based on this pilot study were designed to elucidate the mechanisms of these effects and partition their contributions to the success of seeding procedures (Barbeau et al.[2]).

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Offshore expansion of spawning of the Japanese sardine, *Sardinops melanostictus*, and its implication for egg and larval survival

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Abstract: Spawning grounds of the Japanese sardine, *Sardinops melanostictus*, expanded from the waters above the continental shelf along the Pacific coast of Japan to the oceanic waters around the Kuroshio current during a period of population increase in the first half of the 1980s. Spawning grounds increased from 150 000 km^2 in 1978 to 290 000 km^2 in 1990. The annual average density of egg distribution over the entire spawning grounds increased from 1500·m^{-2} in 1978 to 19 400·m^{-2} in 1986. Percent survival in about the first 10 days of life (from fertilization to the end of the first-feeding stage) increased through the 1980s. Increases in egg abundance and survival were related to the offshore expansion of the spawning grounds into the waters around the Kuroshio axis, which are considered to provide sardine eggs and early larvae with optimal rearing conditions.

Resumé : Les aires de ponte du pilchard du Japon (*Sardinops melanostictus*) se sont étendues, pendant une période d'accroissement de la population survenue dans la première moitié des années 1980, des eaux du plateau continental le long de la côte pacifique du Japon jusqu'aux eaux océaniques situées aux alentours du courant du Kuroshio. La superficie des frayères est passée de 150 000 km^2 en 1978 à 290 000 km^2 en 1990. La densité annuelle moyenne de la distribution des oeufs sur l'ensemble des frayères est passée de 1500·m^{-2} en 1978 à 19 400·m^{-2} en 1986. Le pourcentage de survie pendant les 10 premiers jours de vie environ (de la fécondation à la fin du stade de début de l'alimentation) s'est accru tout au long des années 1980. L'augmentation de l'abondance des oeufs et de la survie est en rapport avec l'expansion vers le large des aires de ponte, jusque dans les eaux qui entourent l'axe du Kuroshio, où les oeufs et les larves de pilchard aux premiers stades semblent trouver des conditions optimales de développement.
[Traduction par la Rédaction]

Introduction

The Japanese sardine *Sardinops melanostictus* experienced two periods of high abundance in the current century, with one peak in the 1930s (Kurita 1957) and one in the 1980s. Total catch of the sardine in the northwestern Pacific by Japan, Russia, and Korea (Fig. 1) was 0.02 million tonnes (t) in 1970 (Food and Agriculture Organization of the United Nations 1975). The catch increased through the 1970s and 1980s and peaked at 5.4 million t in 1988, but it has been declining since 1989 and fell to 2.4 million t in 1992 (Food and Agriculture Organization of the United Nations 1994).

Spawning grounds of sardines are known to expand with population increases and contract with declines. Lluch-Belda et al. (1989) summarized expansions and contractions of spawning grounds of *S. melanostictus* in Japanese waters, *Sardinops sagax* in Chilean waters, and *Sardinops ocellatus* off South Africa. Lluch-Belda et al. (1992) described changes in the area of the spawning grounds of the California sardine *Sardinops caeruleus* and Chilean *S. sagax*. Spawning of the Japanese sardine was restricted within coastal waters above the continental shelf along the Pacific coast of western and eastern Japan during 1949–1951 when the population was small (Nakai and Hattori 1962). The spawning grounds expanded offshore in the 1980s when the population was large (Kuroda 1988; Kawai 1994). Kikuchi et al. (1992) reported that maximal annual egg densities were recorded from waters offshore of the Kuroshio axis during 1983–1990.

The dominant Kuroshio current, the axial velocity of which is faster than 2 knots (1 knot = 1.853 km·h^{-1}) (Taft 1972), flows eastward along the Pacific coast of Japan and strongly influences physical and biological environments there (Kobayashi and Kuroda 1991; Nakata et al. 1994). Offshore expansion of the spawning grounds is considered to have caused changes in environments where sardine eggs were released that could have influenced survivorship in egg and early larval stages. This paper describes and analyzes offshore

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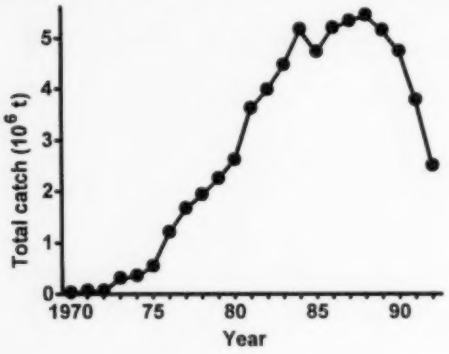
Fig. 1. Total catch of Japanese sardine by Japan, Russia, and Korea in the northwestern Pacific (from Food and Agriculture Organization of the United Nations 1975, 1979, 1984, 1994).



expansion of spawning grounds of the Japanese sardine along the Pacific coast of Japan during the period of radical population changes in 1978–1992. Survivorship from the egg stage to the end of the first-feeding stage was estimated and interpreted in relation to expansion of the spawning grounds.

Materials and methods

Egg and larval census statistics of the sardine off the Pacific coast of Japan between 1978 and 1992 were used in this study. In the censuses, 4262–4855 ichthyoplankton samples were collected each year by towing three types of conical or cylindrical-conical nets of 45 or 60 cm inside mouth diameter, and 0.335-mm mesh aperture. Each net was equipped with a flow meter for standardizing the volume of water strained. As sardine eggs and larvae are distributed in the upper 100 m of the water column (Konishi 1980), the net was retrieved vertically at 1 $m \cdot s^{-1}$ from 150 m depth or from the bottom at stations shallower than 150 m. Sardine eggs and larvae were sorted from the tow samples, and larvae were classified into yolk-sac or feeding larvae under a dissecting microscope. Total lengths (TL) of feeding larvae were measured to the nearest 0.1 mm and assigned to one of the following 2-mm size-classes: <6.0, 6.0–7.9, . . ., 18.0–19.9, and >20.0 mm. Distribution densities (number per square metre) of eggs, yolk-sac larvae, and feeding larvae of the various size-classes were compiled monthly in terms of 30′ latitude × 30′ longitude squares by averaging the densities of eggs or larvae collected by tows in each square in a month. To analyze egg abundance and distributions of spawning grounds in relation to the Kuroshio current, we used data from the waters along the Kuroshio current between 130 and 143°E and between 28 and 36°N.

The area of sardine spawning grounds in a given year was calculated by summing the areas of 30′ longitude × 30′ latitude squares positive for egg collection during October of the previous year to September of the year in question.

Offshore expansion of spawning was analyzed for the main spawning months, February and March (Mori et al. 1988), by categorizing the waters along the Kuroshio current into three zones: 30′ × 30′ squares at least in part on the continental shelf shallower than 200 m were assigned to the continental shelf zone; those whose centers were located inshore of the Kuroshio axis excluding those in the continental shelf zone constituted the inshore zone; and those whose centers were located offshore of the axis were assigned to the offshore zone. Egg abundance in the continental shelf zone in month j, E_{1j}, was calculated by summing egg abundance in squares assigned to the zones.

$$E_{1j} = D_j \cdot \sum_{i=1}^{k} \left(\frac{\bar{x}_{ij}}{I_{ij}} \cdot a_i \right)$$

where D_j is the number of days in month j, $\bar{x}_{ij}$ is the average density of egg distribution in the ith square in month j, and a_i is the area of the ith square (in m^2). I_{ij} is the egg incubation time (in days) in the ith square in month j, which was calculated by Arrhenius' equation using the average sea surface temperature, $\bar{t}_{ij}$, of all of the net tow stations weighted by egg density (Watanabe 1983).

$$I_{ij} = 9.86 \cdot \exp\left(\frac{21\,900}{2} \cdot \frac{1}{\bar{t}_{ij} + 273} \right) \cdot 10^{-17}$$

Egg abundances in the inshore zone, E_{2j}, and in the offshore zone, E_{3j}, in month j were similarly calculated. Total egg abundance in month j, E_j, is the sum of the abundances of the three zones.

The latitudinal center of the spawning grounds in February and March was calculated in reference to the location of the Kuroshio current axis. The distance of the spawning center (in km) from the Kuroshio axis in month j, G_j, was calculated by summing the distances of the center of each 30′ × 30′ square from the nearest Kuroshio axis weighted by egg abundance of the square.

$$G_j = \frac{D_j}{E_j} \cdot \sum_{i=1}^{m} \left(\frac{\bar{x}_{ij}}{I_{ij}} \cdot a_i \cdot d_{ij} \right)$$

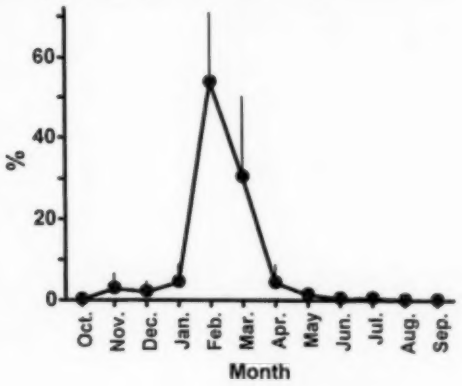
where d_{ij} is the distance (km) of the center of the ith square from the nearest Kuroshio axis in month j. Locations of the Kuroshio axis were determined from the biweekly current profile published by the Maritime Safety Agency (1978–1992). We used the location of the axis in the second half of February as that for February, and the location in the first half of March as that for March. The squares offshore of the axis were expressed as positive values and those in the inshore side as negative. The center was calculated as egg abundance weighted average distance of the centers in February and March.

A feeding larva of 6.0–7.9 mm (preserved TL) was estimated to be 2.4–6.4 days after the initiation of feeding and was assumed to be at the end of the first-feeding stage (Watanabe et al. 1995). The abundance of feeding larvae 6.0–7.9 mm in TL standardized for size-class duration in month j, F_j, was calculated as in Watanabe et al. (1995), using a duration of 4 days.

$$F_j = \frac{D_j}{4} \cdot \sum_{i=1}^{n} \left(\bar{y}_{ij} \cdot a_i \right)$$

where $\bar{y}_{ij}$ is the average distribution density (m^{-2}) of feeding larvae of 6.0–7.9 mm TL in the ith square in month j. The

Fig. 2. Fifteen-year average (+SD) of monthly egg abundance as a percentage of annual abundance.



percent survival from egg to the end of the first-feeding stage was calculated by taking a ratio of the abundance of post first-feeding larvae in February and March to that of eggs.

Results

The 15-year average of monthly egg abundance in February as a percentage of annual abundance was 55.4 ± 18.5% (mean ± SD) and that in March was 30.3 ± 20.0% (Fig. 2). These were the two main spawning months of the sardine on the Pacific side of Japan in the 15 years studied.

The spawning grounds extended from the west of Kyushu to the east of Boso Peninsula along the Kuroshio current (Fig. 3). Sardine eggs were distributed mostly on the continental shelf along the Pacific coasts in 1979, the year of the minimum egg abundance among the 15 years studied. The spawning grounds moved to the waters off western Japan and expanded offshore toward the Kuroshio axis in 1986, the year of maximal egg abundance. In 1992, sardine eggs were distributed along the Kuroshio axis off western and eastern Japan.

The area of the sardine spawning grounds expanded steadily from 148 000 km^2 in 1978 to 277 000 km^2 in 1987 (Fig. 4). The area decreased to 229 000 km^2 in 1989 then peaked at 290 000 km^2 in 1990, and shrank to 205 000 km^2 in 1992. Spawning area (in thousands of square kilometres) (Y) and annual egg abundance (in trillions) (X) were positively correlated, according to the expression $Y = 60.9X^{0.181}$ ($r^2 = 0.524$, $n = 15$, $P < 0.01$) (Fig. 5). The average density of egg distribution over the entire spawning grounds, calculated as annual egg abundance per square metre of spawning grounds, increased with egg abundance from 1500 to 19 400·m^{-2}·year^{-1} during 1979–1986. The egg density (Y) was highly correlated with the total egg abundance (X): $Y = 778 + 3.74X \times 10^{-12}$ ($r^2 = 0.961$, $n = 15$, $P < 0.001$) (Fig. 6). The enormous egg productions in the second half of the 1980s resulted from the increases in spawning area as well as in the density of egg distribution.

Egg abundance in the main spawning months, February and March, stayed below 700 trillion during 1978–1983. Abundance in the continental shelf zone, the inshore zone, and the offshore zone in these years was 251 ± 83, 98 ± 136, and 84 ± 55 trillion, respectively (Fig. 7). On average, 64% of eggs in the main spawning months occurred in the continental shelf zone in these years.

Egg abundance in the main spawning months began to

Fig. 3. Annual egg distributions in 1979, 1986, and 1992 along the Pacific coast of Japan. Solid circles indicate annual egg abundance in a square (in trillions), standardized for incubation time. The solid line indicates the location of the Kuroshio current axis in the month of maximal egg abundance in the year: the second half of February for 1979, 1981, 1992, and the first half of March for 1986. Egg census data in the areas west of 130°E or north of 36°N were not used for the analyses in this study.

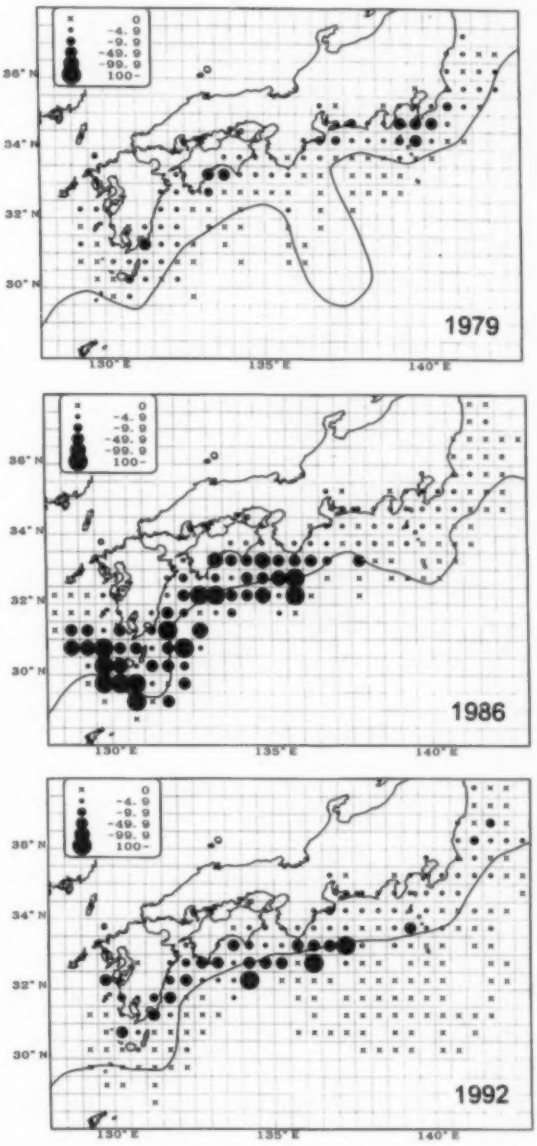


Fig. 4. Changes in area of annual spawning grounds of sardine along the Kuroshio current.

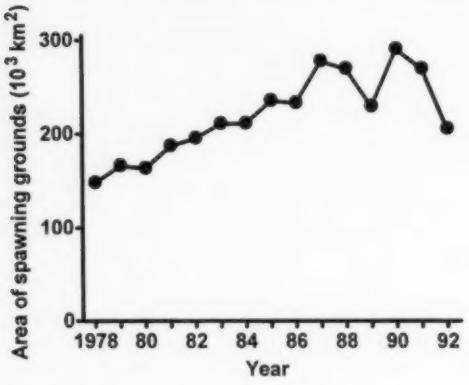


Fig. 5. Regression of area of annual spawning grounds of sardine on annual egg abundance.

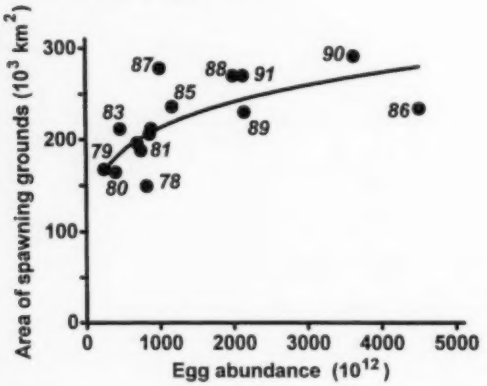
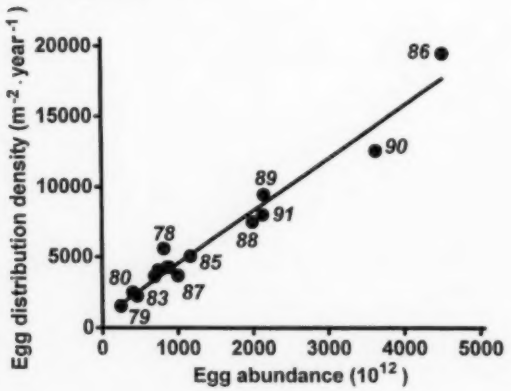


Fig. 6. Linear regression of density of egg distribution on annual egg abundance.



increase after 1984 and peaked in 1986 and 1990 (Fig. 7). Although abundance in the continental shelf zone during 1984–1992 was 291 ± 235 trillion, the same level as that

Fig. 7. Egg abundance in the main spawning months, February plus March, in the continental shelf (C), inshore (I), and offshore (O) zones. The vertical bar represents the SD of the sum of the abundances in the three zones.

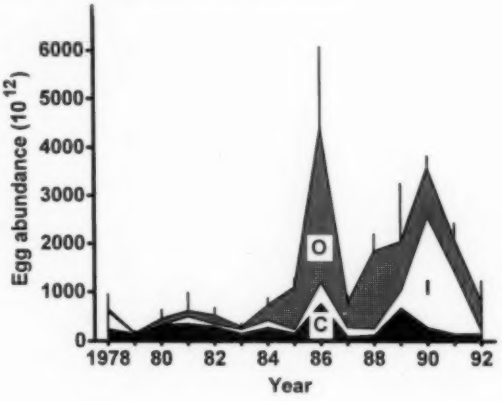
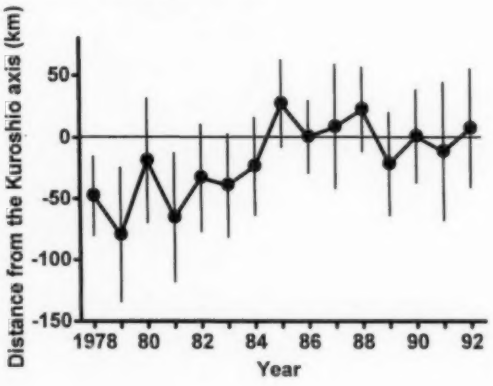


Fig. 8. Mean distance (±SD) of the spawning ground center from the Kuroshio axis in the main spawning months. The center offshore of the axis is expressed as a positive value and that inshore as a negative value.



during 1978–1983, the abundance in the offshore zone first exceeded that in the continental shelf zone in 1984 and increased remarkably during 1984–1992 to 1070 ± 819 trillion, which was more than 10 times higher than that during 1978–1983 (84 ± 55 trillion). In 1990 and 1991, abundance in the inshore zone (2294 and 1301 trillion) constituted more than 60% of the total abundance.

The latitudinal center of the spawning grounds in the main spawning months was located 79.5 km inshore of the Kuroshio axis in 1979 (Fig. 8). It gradually moved toward the Kuroshio axis with interannual fluctuations and reached 27.1 km offshore of the axis in 1985. The center stayed within 25 km of the axis after 1986. Adult sardines spawned in the waters around the Kuroshio axis in the second half of the 1980s and the beginning of the 1990s. The offshore movement of the spawning ground center in the second half of the 1980s was caused by intensive egg production in the waters offshore

Fig. 9. Percent survival between the egg stage and the end of the first-feeding stage in the main spawning months.

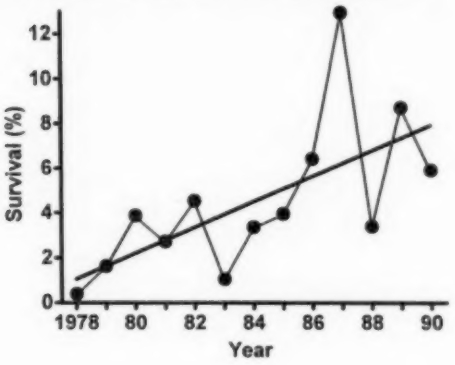
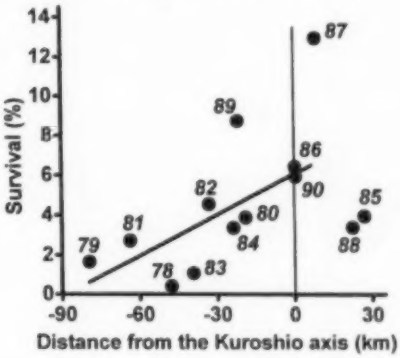


Fig. 10. Correlation of percent survival with the distance of the spawning ground center from the nearest Kuroshio axis. The center offshore of the axis is expressed as a positive value and that inshore as a negative value.



of the continental shelf zone while production in the continental shelf zone remained stable (Fig. 7).

Survival between the egg and post first-feeding stages was 0.4 and 1.6% in 1978 and 1979, respectively. It increased to the range of 1.0–5.0% during 1980–1985. Although the increasing trend in survivorship (Y) over 13 years (X) was significant ($Y = 6.07 + 0.0687X$, $n = 13$, $r^2 = 0.460$, $P < 0.02$) (Fig. 9), survival became more variable after 1986.

Survival increased with the move of the spawning ground center toward the axis in the inshore waters of the Kuroshio (Fig. 10). The relationship between distance (X) and survival (Y) in the years when the center was located inshore or on the Kuroshio axis can be expressed by $Y = 6.07 + 0.068X$ ($r^2 = 0.406$, $n = 10$, $P < 0.05$). Survival was low in 1985 and 1988 when the centers were located further than 20 km offshore of the axis.

Discussion

Japanese sardine spawned mainly in February and March along the Pacific coast of Japan during the period of high population. Some 86% of annual egg production was concentrated in these months. In the years of low sardine population, 1949–1951, spawning occurred between February and May with a peak in April (Nakai and Hattori 1962). In the west of Kyushu and in the Sea of Japan, the main spawning months were March, April, and May (Ito 1961; Shojima 1985). Spawning of the Japanese sardine is restricted to a relatively short period from late winter to spring, which contrasts with three other species of *Sardinops* distributed in other waters. The California sardine *S. caeruleus* spawns almost all year round with a high season from January to June (California Cooperative Sardine Research Program 1952). The Chilean sardine *S. sagax* has two spawning seasons, July–September and December–January (Lluch-Belda et al. 1992). The South African sardine *S. ocellatus* has two spawning seasons as well, the winter–spring and summer–autumn seasons (Le Clus 1988, 1992). Temporal concentration of the spawning activities in February and March seems to be connected with spatial expansion of spawning grounds and an increase in the density of egg distribution on the Pacific side of Japan in the years of maximal population in this study.

Egg abundance on the continental shelf was stable through the years of offshore expansion of spawning (Fig. 7). Coastal spawning of the Japanese sardine was reported during the years of small population (Nakai and Hattori 1962). Lluch-Belda et al. (1992) identified shrunken spawning areas off Punta Eugenia and Antofagasta refuge areas for California and Chilean sardines, respectively, when the populations were minimal. *Sardinops* species are assumed to be originally coastal spawners.

During periods of population increase, the spawning grounds of California and Chilean sardines expanded north- and south-wards along the west coasts of North and South America (Lluch-Belda et al. 1989, 1992). This expansion differs from the offshore expansion of spawning grounds in the case of the Japanese sardine: an average of 36% of sardine eggs were produced offshore of the continental shelf until 1983 but this value increased to an average of 83% during 1984–1992. Year-classes of the sardine on the Pacific side of Japan were strong in 1980 and 1981 (Wada 1988) and they first matured at the age of 3 (Watanabe 1987). The spawning population became large in 1984 when both of the year-classes became mature and dominant in the spawning population. Egg abundance in the offshore zone first exceeded that in the continental shelf zone in 1984 (Fig. 7). The increase in spawning population in 1984 caused the offshore expansion of spawning activities from coastal to oceanic waters. Another strong year-class was established in 1983 (Wada 1988) and they first matured in 1986, which is considered to have accelerated oceanic spawning after 1986.

Wolf and Smith (1985) formulated a relationship between spawning area and spawning biomass of *S. caeruleus*. Smith (1990) attempted to estimate the spawning biomass of the sardine by using a proportion of ichthyoplankton net tows positive for sardine eggs, and found that the spawning area would be a useful index for monitoring a sardine stock at high levels. The spawning area of the Japanese sardine positively correlated with egg abundance. A rough estimate of egg abundance, and therefore a spawning biomass, can be obtained from the allometric relationship illustrated in Fig. 5.

Sea surface temperatures of the Kuroshio were 19–20°C

(Maritime Safety Agency 1978–1992). Eggs spawned in the Kuroshio can be transported eastward about 100 nautical miles (1 nautical mile = 1.853 km) (three 30′ squares) by a current as fast as 2 knots (Taft 1972) for the 2-day incubation; this could have inflated our spawning area estimates in the years of offshore spawning. As the width of the Kuroshio is about 60 nautical miles (2–3 squares), overestimation could reach 6–9 squares, which constituted 5–10% of the total spawning area.

We analyzed sardine spawning by dividing the spawning grounds into three zones. Thus, we need to consider whether or not eggs are retained in their zones of origin to validate our results of offshore expansion of sardine spawning. Kobayashi and Kuroda (1991) demonstrated by numerical model experiments of entrainment to and detrainment from the Kuroshio current that 49–59% of the eggs (larvae) spawned in the coastal region were retained in the region on the 5th day after spawning. The egg incubation times calculated from annual average sea surface temperature weighted by egg density (H. Zenitani, Y. Watanabe, and R. Kimura, unpublished data) were in the range of 2.4–1.8 days. Assuming that the daily rate of egg entrainment to the Kuroshio current from the coastal region is constant, and that egg incubation time is 2 days, 87–90% of 1-day-old eggs and 75–81% of 2-day-old eggs are estimated to be retained in the coastal region. Similarly, retention of eggs spawned in the Kuroshio region south of Kyushu is calculated to be 88–90% for 1-day-old and 77–81% for 2-day-old eggs. Kasai et al. (1992) modelled offshore transport of eggs and larvae by wind-driven surface current. From their 20-day simulation period, we estimated that only a small fraction of the eggs can be transported between the three zones in this study in 2 days. Hence, the estimated egg abundances in the three zones in this study are considered to represent egg production in the zones.

The offshore expansion of spawning might have mitigated the density-dependent reduction of spawning activity of individual fish during peak years of the sardine population. Tsuruta and Hirose (1989) reported decreases in batch fecundity and spawning frequency with increasing density of adult Japanese anchovy *(Engraulis japonicus)* in captivity. If the offshore expansions of spawning of the sardine had not occurred, egg density could have increased in an exponential manner and resulted in high mortality related to egg density, because of such factors as cannibalism and predation (Bailey and Houde 1989; Brodeur et al. 1991).

Survival rates between the egg stage and the end of the first-feeding stage tended to increase through the years studied, and this trend seemed to be related to the offshore expansion of the spawning (Fig. 10). H. Zenitani, Y. Watanabe, and R. Kimura (unpublished data) showed that the spawning temperature increased with the offshore move of the spawning grounds from 17.3 to 19.8°C during 1978–1988. Zenitani (1995) found that sardine larvae were larger at the yolk exhaustion stage when eggs and yolk-sac larvae were incubated at 19.0°C than when they were incubated at 15.0 or 23.0°C. Survival of larger larvae at the end of the yolk-sac stage can be higher than that of smaller larvae (Blaxter and Hempel 1963). High survival in the years when the spawning ground center was located around the Kuroshio axis could be due partly to high spawning temperature.

Another possible cause of high survivorship of early larvae is that Kuroshio waters provide a favorable food environment. The oceanic front between coastal and Kuroshio waters is highly productive for zooplankton (Nakata 1990) and is an important feeding area for sardine larvae (Nakata et al. 1991). Nakata (1995) demonstrated that more than 68% of sardine larvae collected in the Kuroshio region in daytime had food organisms in their guts, while more than 65% of larvae collected in the waters inshore or offshore of the Kuroshio region had empty guts. Kimura et al. (1996) examined the nutritional condition of first-feeding larvae collected in the Kuroshio region by RNA–DNA analyses and found that more than 90% of them were in a healthy condition. From these observations and analyses together with the positive correlation between percent survival and the distance of the spawning ground center from the Kuroshio axis in this study, the Kuroshio region is considered to provide sardine larvae with a nursery area that improves the survivorship of eggs and early larvae of the sardine.

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Persistent pollutants in a salmon population (*Salmo salar*) of the southern Baltic Sea

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Abstract: Persistent pollutants in an Atlantic salmon (*Salmo salar*) population spawning in a river of southern Sweden were investigated. The population was characterized by a high growth rate. Some males reached 20–30 kg after 3 to 4 years in the sea. The fat content of migrating salmon varied by one order of magnitude and was the most significant correlate of pollutant concentrations. No relationships were recorded between fat content and gender, age (weight, length), year at sea, or different measures of condition. The reasons for this may be the varied evolutionary strategies for maximizing reproductive output; male salmon may enter the spawning river as small grilse and spawn opportunely, or migrate at a larger size, when they can hold spawning territories in the river. Females have a greater and more uniform size, and spend more energy on gonadal products. The varying fat content of individual fish may also be attributed to foraging in different areas of the Baltic and thereby to migration distances as well as foraging strategies. When pollutant levels were normalized for fat content, other factors such as age (weight, length) were shown to be important for uptake; older fish had higher levels of pollutants than younger ones.

Résumé : Nous avons examiné les polluants rémanents dans une population de saumon atlantique (*Salmo salar*) frayant dans une rivière du sud de la Suède. Cette population se caractérisait par un taux de croissance élevé. Certains mâles atteignaient 20–30 kg après 3 ou 4 ans en mer. La teneur en graisse des saumons en migration variait d'un ordre de grandeur, et constituait le corrélat le plus significatif des concentrations de polluants. On n'a observé aucune relation entre la teneur en graisse et le sexe, l'âge (poids, longueur), le nombre d'années en mer ou différentes mesures de la condition. Cela peut s'expliquer par les diverses stratégies d'évolution qui visent à maximiser la ponte; les mâles peuvent entrer dans la rivière de ponte quand ils sont encore des madeleineaux et se reproduire quand l'occasion se présente, ou encore attendre d'être plus grands pour migrer, et occuper alors un territoire de fraye dans la rivière. Les femelles sont de taille plus grande et plus uniforme, et dépensent davantage d'énergie pour le développement des gonades. La teneur variable en graisse des poissons pris individuellement peut aussi être attribuée au fait qu'ils s'alimentent dans différentes régions de la Baltique, et donc aux distances parcourues en migration aussi bien qu'aux stratégies de recherche de nourriture. Une fois normalisés les niveaux de pollution en fonction de la teneur en graisse, d'autres facteurs comme l'âge (poids, longueur) sont apparus importants dans l'absorption de polluants; les poissons âgés en présentaient des concentrations plus grandes que les jeunes.
[Traduit par la Rédaction]

Introduction

The Atlantic salmon (*Salmo salar*) is distributed in Europe from the Arctic circle in the north to Portugal in the south. It is an anadromous fish that spawns in rivers, and the young migrate to the sea as smolts in their 2nd to 4th year of life, depending on the latitude and the availability of nutrients. Salmon are pelagic feeders, and prey mainly on herring (*Clupea harengus*) in the open Baltic sea. The growth rate increases in the sea, and the fish return to spawn within 1–4 years. This life cycle makes these fish vulnerable to disturbances during reproduction (Gross 1987), and the abundance of salmon has decreased as a result of reduced water quality and construction of hydroelectric dams.

Within the Baltic Sea, Atlantic salmon have a high commercial value and the fishing pressure is great both in the open sea and in coastal waters during the spawning migration. Additionally, sport fishing is common in the rivers where salmon still spawn. The majority of the salmon within this area are cultivated by compensatory hatcheries on the rivers, which rear and release smolts. During the last decade there has been a dramatic increase in mortality in cultivated salmon fry, initially characterized by edema, hemorrhages, and ataxia known as the M74 syndrome (Norrgren et al. 1993). The

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syndrome is restricted to hatcheries situated on Baltic rivers; salmon on the west coast of Sweden are not affected. The reasons for the increased mortality are not known, but it has been speculated that the effect is caused by persistent pollutants, by decreasing genetic variability of the salmon stock (as the cultivated fry originate from a few females), or by vitamin deficiency. A similar situation has been recorded for young salmonids in Lake Michigan and Lake Ontario, where lipid-soluble substances such as polychlorinated biphenyls (PCBs) and chlorinated dioxins have been shown to interfere with embryogenesis (Walker et al. 1991).

The load of persistent pollutants in the Baltic Sea is high and comparable to that of the Great Lakes in North America (Thomann 1989; Borgmann and Whittle 1992). Studies on persistent pollutants in the Great Lakes have focused on predatory fish, such as lake trout (*Salvelinus namaycush*). This is not the case for the Baltic, where studies have concentrated on fish lower in the food chain and time trends for PCB and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) concentrations are described for zooplanktivorous herring (Olsson and Reutergård 1986). However, the exposure to persistent pollutants is highest among piscivorous predators and especially in diadromous fish that deposit fat in muscular tissue (Larsson et al. 1991). The few analyses performed on Baltic salmon (Andersson et al. 1988; Norrgren et al. 1993) indicate levels of persistent pollutants at least two orders of magnitude higher than for cod (*Gadus morhua*, von Westerhagen et al. 1987) that contain low amounts of fat. Pollutants detected in Baltic salmon include PCBs, DDT with metabolites, chlorinated dioxins and dibenzofurans, hexachlorocyclohexanes, chlordane, and toxaphene (Andersson et al. 1988; Norrgren et al. 1993).

Pollutants dissolved in fat deposits may be released during migration (Larsson et al. 1991). Fishes that spawn only once (like European eel, *Anguilla anguilla*, and some North American salmon species) will accumulate persistent pollutants throughout their life. Salmon that spawn once or a few times during life may also accumulate high levels of pollutants. The high pollutant load may affect the animal when under stress; for instance, during migration or starvation, as the fat is used and the pollutants are redistributed to vital organs, behavioural changes or disturbances in reproduction may result. Lethal effects of pollutants have been shown in starving water-dwelling birds (Krom 1986), and impairment of reproduction (M74 syndrome) has been observed in Baltic salmon and in salmon in the Great Lakes (Stauffer 1979; Morrison et al. 1985; Walker et al. 1991; Norrgren et al.1993). The recruitment of eel fry (*Anguilla anguilla* and *Anguilla rostrata*) has decreased during the last decades, possibly as a result of pollutants (Larsson et al. 1991; Hodson et al. 1994).

The variation in the levels of persistent pollutants within a population is largely due to physiological and ecological factors, like age, gender, fat content, food web structure, and habitat. For North American species such as chinook salmon, *Oncorhynchus tschawytscha* and lake trout, *Salvelinus namaycush*, levels of persistent pollutants increase with age for both males and females (Borgmann and Whittle 1992; Miller 1994). Fast growth results in a biomass dilution of persistent pollutants (Thomann 1989; Thomann et al. 1992; Borgmann and Whittle 1992), and slowly growing fish, therefore, have higher levels of pollutants. This phenomenon was shown for Arctic char (*Salvelinus alpinus*), where the population of a single lake was divided into normally growing fishes and a dwarf form (Hammar et al. 1993). The slowly growing dwarfs had significantly higher levels of pollutants than the normally growing fishes. Fish species with a high fat content have greater total amounts of lipophilic pollutants (Borgmann and Whittle 1992). For a population of European eel inhabiting a lake (only females, as the males stay in coastal areas in the sea) in southern Sweden, fat content and levels of PCB and DDT increased with age (Larsson et al. 1991). Levels of pollutants were also inversely correlated with the instantaneous growth rate.

Fishes with low fat content show quite another picture of pollutant accumulation. Northern pike (*Esox lucius*) contain less than 0.5% fat in muscle tissue, and levels of PCB, DDT, and lindane (γ-hexachlorocyclohexane) decrease with age in females but increase in males (Larsson et al. 1993). Female fish eliminate pollutants yearly by spawning eggs that contain about 5% of fat (on a wet weight basis) and constitute almost 15% of the body weight. This depuration process does not occur to the same extent with milt in males.

A high fat content in fishes is often correlated with long spawning or foraging migrations in which the fat is used for gonadal growth or as an energy source. Fat content has also been found to be a regulator of sexual maturity and spawning migration in Atlantic salmon (Thorpe 1986; Rowe et al. 1991), European eel (Larsson et al. 1990), Arctic char, and whitefish (*Coregonus laveretus*, Reshetnikov et al. 1970). At a certain species-dependent content of fat, hormone systems are activated that trigger these processes. Anguillids may contain up to 30% fat in muscle tissue (on a wet weight basis), while the fat content of salmonids seldom exceeds 20%. During migration the fat content of muscle tissue decreases when energy is released for swimming, sexual maturation, and gonadal development. During the 1100-km migration of some sockeye salmon (*Oncorhynchus nerka*) populations the fat content of muscle tissue decreased by 69% for males and 82% for females (Henderson and Tocher 1987). Less than 0.5% of this decrease in fat was attributed to development of testes, while about 8% was used for development of ovaries. For chum salmon (*Oncorhynchus keta*), fat content decreased from 4–12% to about 1% during 2 months when the fish moved from the open sea to coastal areas and approached sexual maturity. Redistribution mechanisms of lipophilic, persistent pollutants from muscular tissue to tissues such as the gonads during the migration are mainly unknown.

The objectives of this study were to examine the distribution of persistent pollutants within a salmon population of the southern Baltic, to establish if accumulation of the substances is gender or age dependent, and to determine whether other variables such as growth rate, fat content, or food choice are important. Another goal was to examine if different foraging areas in the Baltic (open sea versus coastal areas) were reflected in the pollutant matrix of the fish (i.e., whether the compositions of persistent pollutants differed). Finally, the pollutant matrix of stationary, freshwater fish of southern Sweden (northern pike) was compared with that of the migratory salmon, to establish if certain pollutants were present in salmon but not in pike. If such a difference was recorded, it might explain the M74 syndrome of salmon. The overall objective of the study was to obtain background information on

persistent pollutants in a salmon population and on ecological and physiological variables for later studies of M74 (fry mortality).

Materials and methods

Sampling

Salmon were caught from July 15 to August 21, 1993 (salmon spawn in October and November in this area), by fyke-net in the mouth of the River Mörrum (mean discharge 29 m^3/s) in southern Sweden. The total weight (to the nearest gram), length (to the nearest millimetre), and gender were determined and the salmon were frozen (–20°C). A muscle sample of about 100 g was taken from behind the neck, and a 5-g subsample was used for analysis of fat and persistent pollutants. Scales (10–20) from the side of the fish (above the lateral line, below the dorsal fin) were removed and used for age determination, back-calculations of growth, and records of earlier spawning. The salmon were aged from annuli on dried scales under reflected light. The hatching date of fish was assumed to be in March. Growth of salmon was back-calculated from the body length – scale radius relationship, and measurements of annulus radii on scales (Bagenal 1978). If fish had spawned earlier, this was recorded as erosion marks on the scale.

Extraction, gas-chromatographic analysis, and fat

About 5 g of fish muscle was chopped coarsely, weighed, and freeze dried. The dry sample was homogenized, weighed again, and transferred to a glass extraction vial for extraction with acetone (Merck): *n*-hexane (Merck, 10:8 v/v) in a modified Soxhlet apparatus for 3 h. Octachloronaphthalene was used as an internal standard and was added prior to the extraction. A subsample of the extract was used to gravimetrically determine the fat content. The remaining extract was subjected to a water equilibrium to separate the acetone and polar impurities, whereafter the hexane fraction was evaporated in a vacuum centrifuge.

To clean the samples properly, an open column step was performed. A silica gel column was prepared by adding 4 mL of activated silica gel (Merck, 70–230 mesh) soaked with 1 g of concentrated sulphuric acid (99%) (Merck, and (fumic)) to 18 mL of the elution liquid, a mixture of *n*-hexane:dichloromethane (Merck, 95:5 v/v), in a glass column. When this gel was sedimented, 4 mL of silica gel soaked with 1 mL of 1 M K_2CO_3 (aq., pH 11) (Merck) was added. The basic gel adsorbed acidic substances (e.g., fatty acids) and the acidic gel oxidized and retained nonpersistent compounds. To keep the gel in place a porous polypropylene disc was placed on top. The solvent in the packing step was eluted and another 5 mL of elution liquid was added to rinse the column.

The samples were dissolved in *n*-hexane (including rinsing, a total volume of 1.2 mL) and transferred to the silica gel column. Three millilitres of elution liquid was added and the eluate discarded. The eluate from the next addition of 5 mL elution liquid was collected. This fraction contained PCB, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p′*-DDE), *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD, hexachlorobenzene (HCB), and the major part of α-hexachlorocyclohexane (α-HCH) and pentachloroanisole. In a second fraction of 10 mL, other pollutants such as β-HCH and a major part of γ-HCH, toxaphenes, and chlorinated paraffins were collected. The eluate was placed in a vacuum centrifuge and the solvent evaporated after which 400 µL of *iso*-octane (Merck, 4727 p.a.) was added.

Samples were analysed for organochlorine pollutants by capillary-gas chromatography – electron capture detection on a Shimadzu GC-14A equipped with a split–splitless injector. The PCB components were separated on a 20-m DB 5 quartz capillary column (i.d. 0.18 mm, Rtx-5, Restek Corporation) with H_2 (1.5 mL/min) as carrier gas and N_2 (50 mL/min) as make-up gas. The oven temperature was programmed to keep the initial temperature at 80°C for 15 min, then increase it at 4°C/min to 300°C, and hold there for 15 min. The injector and detector temperatures were 80 and 310°C, respectively. The peaks were automatically integrated (Schimadzu C-R5A) and stored on a computer.

PCB components were identified and quantified as domains (with response factors) according to Mullin et al. (1984) and Schulz et al. (1989) by an automatic computer program. Pentachlorobensen was used as a chromatographic standard (to check retention and response). The analytical performance was also regularly checked with PCB standards (Aroclor 1242 and Clophen A60) and different pesticides.

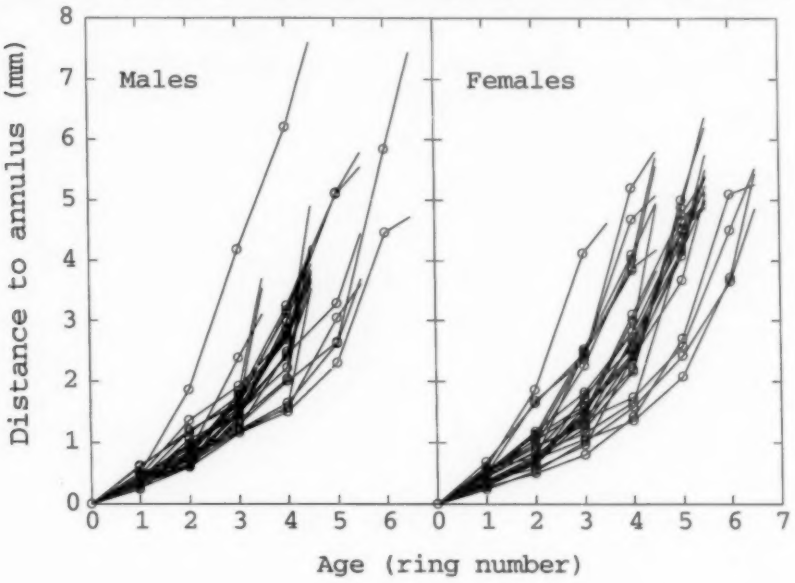
All pollutant concentration values were log-transformed to compute statistical comparisons, owing to the skewed distributions of the values (Newton 1988). The Pearson product-moment correlation test was used if not otherwise stated.

Results and discussion

We tried to reflect the sizes and life histories of the migrating salmon population with our sampling. For males ($n = 25$) this meant that the majority of the fish were small (grilse) with a weight of 2–5 kg, and the remainder were large fish, weighing 11.4–32 kg ($n = 5$). For females ($n = 31$), the mean weight was greater (9.5 kg) and more evenly distributed (SD = 6.5). Most fish had migrated to sea after 2 years in the river, but the population was also represented by fish that had migrated to sea after 1 year (cultivated fish), or after the 3rd or 4th years. The maximum time spent in the sea was 4 years before the salmon returned to the river, and the oldest fish was aged 6+ years. After migrating to the sea, these fish were characterized by a high growth rate (Fig. 1), as exemplified by the biggest male (32 kg). This fish migrated to sea after 1 year and was captured after 3 years of foraging there. It grew at 10.6 kg/year (calculated over the whole sea period). None of the salmon in our sample had spawned previously.

The strongest correlate of levels of persistent pollutants in salmon was fat content. The concentration of ΣPCB (the sum of 57 domains, which generally are composed of 1–2 congeners, Schulz et al. 1989), ΣDDT (*p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD), ΣHCH (α,β,γ-he xachlorocyclohexane), and HCB was significantly correlated to fat content in the muscle tissue ($r^2 = 0.49, 0.55, 0.77$, and 0.66, respectively, $p < 0.01$, Fig. 2). Since the concentrations of all persistent pollutants correlated (with the exception of α- and β-HCH), this was also valid for such compounds as specific PCB domains (congeners) or *p,p′*-DDE. Fat content in salmon showed high variation, between 0.5–12.3% of fresh weight. No relationships were found between fat content and age (weight, length), gender, years spent at sea, or different measures of condition (the simplest being weight/length).

Fig. 1. The relative growth rate of salmon caught in the river mouth of Mörrum. The growth rate was measured as the distance from the centre to the annulus of scales in millimetres, and the weight of an individual can be back-calculated from scale annuli according to the formula $L_n = R_n/R\ (L - L_o) + L_o$, where L_n is length at age n (cm), R_n is scale radius at age n, R is scale radius, L is total length (cm), and L_o is length at the time when primary scales are developed (calculated).



Fat content has been shown to be of major importance in explaining uptake of persistent pollutants, but mainly for explaining interspecific differences. The variation in fat content was similar to that observed in eels (Larsson et al. 1990), although fat content was higher in the latter species. However, fat content varies systematically with age for eels, as the muscle fat is used to store energy for migration to the Sargasso Sea (Larsson et al. 1990). It was, therefore, surprising that the fat content in salmon showed such high, unsystematic variation during their return to their spawning river. There are several possible explanations for this phenomenon, as follows.

(*i*) Male salmon have several evolutionary strategies to maximize reproductive output. They may return from sea at a smaller size (<5 kg), as grilse, and spawn opportunely. The majority of males are grilse. Other males invest in maximum growth and do not return to spawn until they have reached a size at which they are able to hold territories in the river. They are a minor part of the population. Females have a greater and more uniform mean weight and invest a major part of their energy in roe. This heterogeneity in strategy results in large variation in the energy resource, i.e., in the fat content of muscle tissue.

(*ii*) Salmon migrate from different foraging areas in the Baltic Sea, some areas more distant from the home river than others, and therefore utilize varying amounts of fat for the spawning migration.

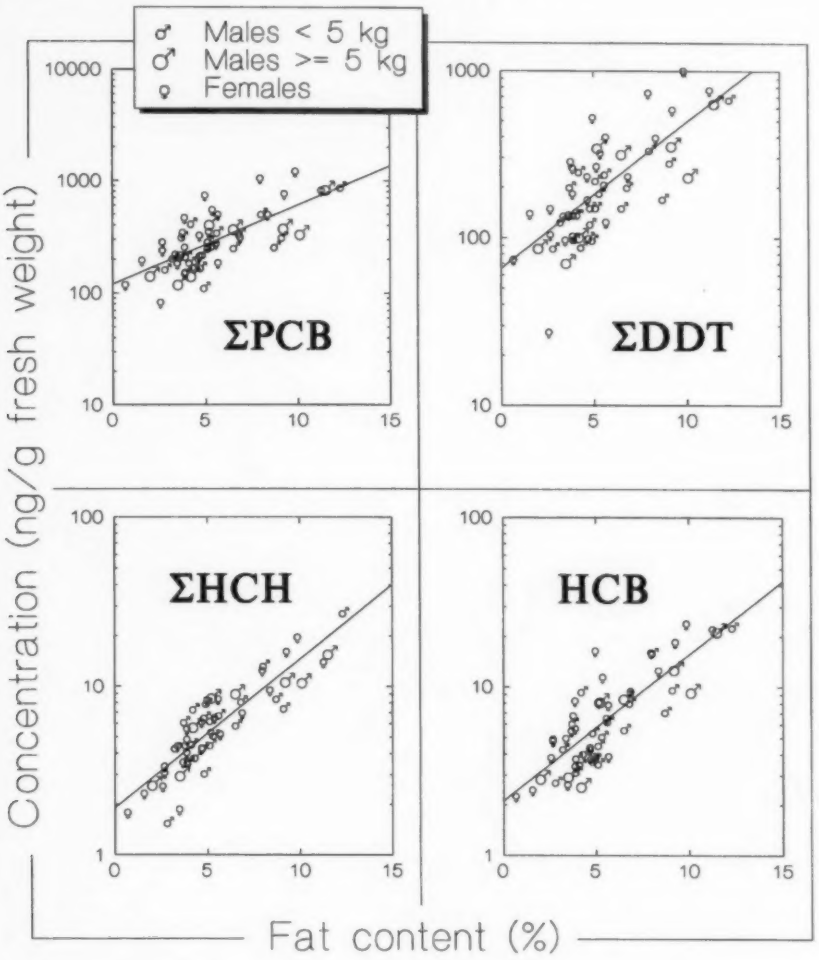
(*iii*) Salmon have different foraging strategies in the Baltic Sea, some feeding mainly on herring while others also use other types of prey, such as other species of fish, larger zooplankton, and benthic invertebrates. When feeding on resources that contain more fat, such as herring, salmon may increase their growth rate and the fat content in muscular tissue. Borgmann and Whittle (1992) showed that fat content and the persistent pollutants in a predator co-vary with the fat content and pollutants in prey. In the Great Lakes of North America, the fat and pollutant content of lake trout varied over years, and was governed by the availability of young or old alewife as food. When the predator was foraging on abundant and older prey that had higher amounts of fat and pollutants, its levels of pollutants and fat increased.

When concentrations of persistent pollutants in salmon were normalized for differing fat content (i.e., transformed to nanograms per gram of extractable fat), other factors were shown to have importance for pollutant uptake. Levels of ΣPCB, ΣDDT, ΣHCH, and HCB correlated with length ($p < 0.01$, but for ΣHCH, $p = 0.04$, Fig. 3). Significant relationships were also recorded for age or weight and concentration of persistent pollutants. The relationships have been shown previously for a number of fish species (Borgmann and Whittle 1992; Miller 1994).

Since none of the salmon had spawned previously, no persistent pollutants were eliminated by spawning. This elimination route may imply that the relationship between age and the concentration of persistent pollutants in females should be negative (Larsson et al. 1993). The importance of this elimination route is enhanced by spawning frequency and an increase in the fat content of the roe compared with other fat deposits in the fish.

The results from studies on fatty fishes that seldom spawn, on fishes containing intermediate levels of fat that spawn infrequently, and on lean fishes that spawn yearly lead to a picture of persistent pollutant load in fish species over lifetime

Fig. 2. The relationship (log $Y = aX + b$) between muscle fat content and concentration of persistent pollutants (ng/g wet weight, log scale) in salmon. For ΣPCB, ΣDDT , ΣHCH, and HCB, the values of a were 0.070, 0.087, 0.088, and 0.087, respectively. The values of b were 2.1, 1.8, 0.3, and 2.8, respectively. The values of r^2 were 0.49, 0.55, 0.77, and 0.66, respectively ($p < 0.01$ in all cases).

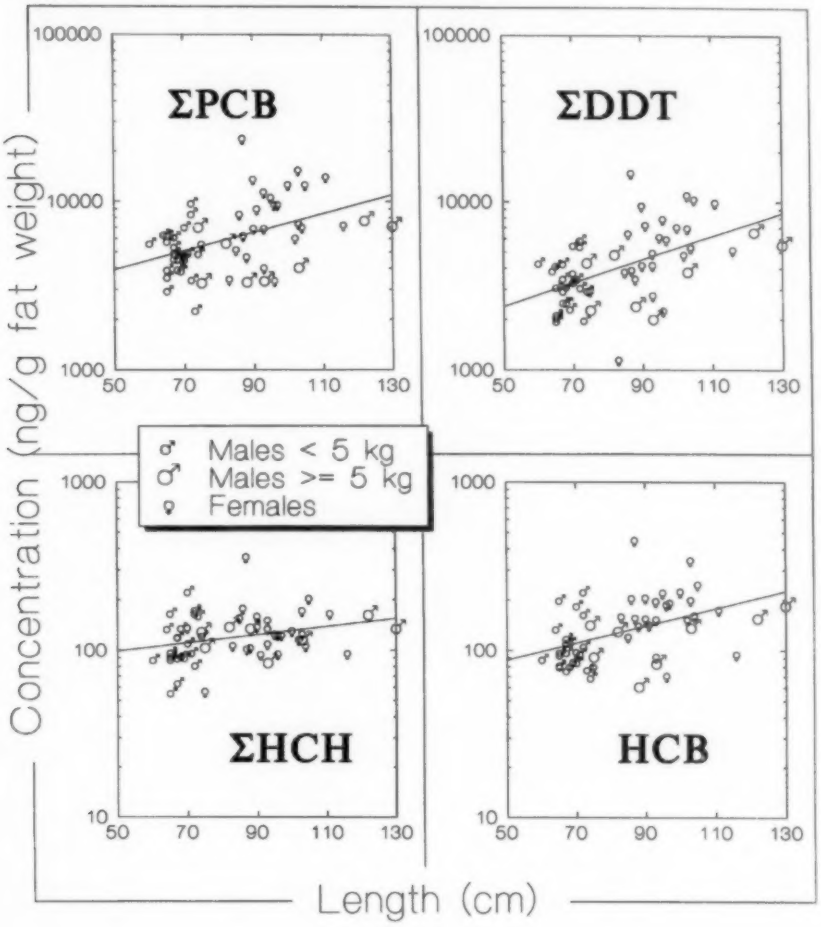


(Fig. 4). Fatty fishes that deposit intramuscular fat (mainly triglycerides), such as eel (Henderson and Tocher 1987; Larsson et al. 1991) and salmon species (Borgmann and Whittle 1992), and that spawn only once or twice in a lifetime, accumulate high amounts of persistent pollutants. These species are mainly diadromous. They have a high fat content in muscle tissue and accumulate the substances throughout their life. If the spawning occurs just once in a lifetime, males and females follow the same positive uptake curve with age.

In lean fish species like pike (Larsson et al. 1993) that contain low amounts of fat in muscle tissue (mainly phospholipids) and high roe lipid concentrations (mainly triglycerides) and spawn yearly, females accumulate pollutants until their first spawning, then concentrations start to decrease as pollutants are eliminated in roe. Males, however, accumulate persistent pollutants over their lifetime. The difference in pollutant concentration between males and females is therefore accentuated, as the fat content and pollutant concentrations in roe are higher than in other parts of the body. Total amounts of lipophilic pollutants are lower than in fat fishes. Uptake of the pollutants is also affected by fat quality, as triglycerides (in fat deposits) have a higher capacity to take up substances than polar lipids like phospholipids (Ewald and Larsson 1994).

Fish between these two extremes accumulate pollutants according to their fat and reproductive strategy. Fish that contain intermediate levels of fat and spawn infrequently, such as char (Hammar et al. 1993), accumulate pollutants over their life, but accumulation is less in females. If the fat content of roe is equal to the content of other parts of the body, elimination through reproduction plays a less important role and consequently if the fat content of roe is high, pollutant

Fig. 3. The relationship (log $Y = aX + b$) between total length of salmon and concentration of persistent pollutants (ng/g extractable fat, log scale). For ΣPCB, ΣDDT, ΣHCH, and HCB, the values of a were 0.006, 0.007, 0.002, and 0.005, respectively. The values of b were 3.3, 3.0, 1.9, and 1.7, respectively. The values of r^2 were 0.19, 0.24, 0.08, and 0.20, respectively ($p < 0.01$ in all cases except $p = 0.04$ for ΣHCH).



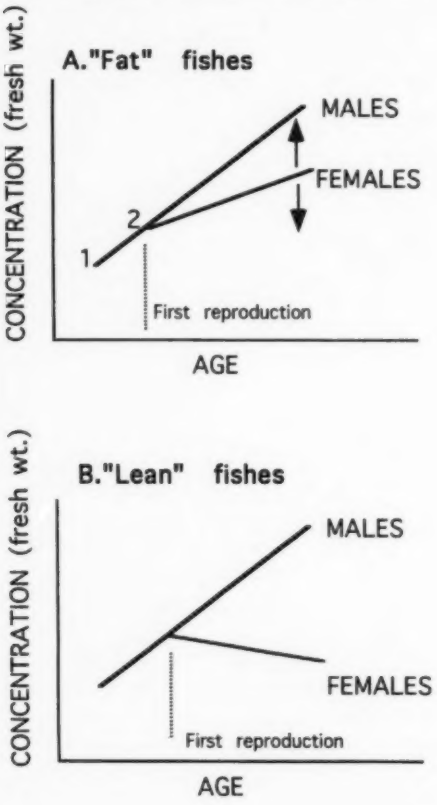
concentrations in females increase at a slower rate. This slower increase leads to larger differences in concentrations between females and males.

For salmon, older (and bigger) females had higher concentrations of persistent pollutants than younger ones. It has recently been shown that the levels of pollutants in muscle tissue are reflected in the roe of salmonids (Miller 1993) and pike (Larsson et al. 1993) by a positive, log-linear relationship. This could mean that fry originating from older females have a lower survival rate, as such fry are exposed to greater pollutant concentrations during hatching and development than fry originating from younger females.

Levels of persistent pollutants in salmon were in the range of 0.11–1.21 µg/g fresh weight for ΣPCB, 0.03–1.00 µg/g for ΣDDT, 2–27 ng/g for ΣHCH, and 2–24 ng/g for HCB. Also, pentachloroanisole and toxaphene were detected in all individuals, but these substances were not quantified. The greatest difference, when comparing the pollutant matrix of salmon with that of freshwater pike of southern Sweden (Larsson et al. 1992), was toxaphene, which was not detected in pike. The proportion of *p,p*-DDT in relation to ΣPCB was also higher in salmon than in pike.

As previously stated, a large proportion of salmon in the Baltic Sea originate from compensatory hatcheries, where adult fish are caught in the river and used for breeding. During 1992, the mortality of fry was 45–90% in different hatcheries (H. Börjesson, Swedish Salmon Research Institute, Älvkarleby, Sweden, personal communication). The high mortality of fry originates from individual females, where all the fry die. Persistent pollutants were indicated as the factor behind this mortality (Norrgren et al. 1993). Relative levels of most persistent pollutants are intercorrelated among individuals of the same species (Larsson et al. 1991, 1992; Williams et al. 1992). Consequently, if a coupling beween levels of pollutants and

Fig. 4. Diagram illustrating the gender-specific uptake of persistent pollutants in (A) fat fishes (*Anguilla anguilla, Salmo salar*) and (B) lean fishes (*Esox lucius, Rutilus rutilus, Perca fluviatilis*). (A) For fishes with a high fat content that spawn once in a lifetime or infrequently, persistent pollutants accumulate in males and females to high amounts throughout life. If a fish spawns just once, males and females follow the same positive uptake curve by age (indicated by upward arrow). For fishes that spawn several times, the uptake curve for females follows the direction of the downward arrow, and the differerence in pollutant levels between males and females increases. Uptake of pollutants in the salmon population is indicated from 1 to 2 (as the fish have not spawned). (B) In lean fish species that contain low amounts of fat in muscle tissue and high concentrations in the roe and spawn yearly, females will accumulate the pollutants until their first spawning, then concentrations will decrease as pollutants are eliminated through spawning. Males, on the other hand, will accumulate persistent pollutants over their lifetime.



increased mortality of fry is suspected, the concentration of one pollutant may be used as an index of the concentration of others. As a result, an analysis of PCB is sufficient to establish whether any of several persistent pollutants may be causing the fry mortality.

Symptoms similar to those of M74 syndrome (yolk-sac fry, characterized by edema, hemorrhages, ataxia, and mortality) have been recorded for salmonids in the Great Lakes (e.g., Stauffer 1979; Morrison et al. 1985; Walker et al. 1991). Giesy et al. (1986) stated that the mortality of chinook salmon hatched before 1970 was 2–3%, then it increased to 19.5% by 1981. The mortality was correlated with high levels of PCB and toxaphene in the roe. The chlorinated hydrocarbons were concentrated in the lipid-rich roe, and the fry received a high dose when the egg yolk was depleted. A similar trend was observed in coho salmon during the 1960s (Johnson and Pecor 1969). High mortality of salmon fry was recorded at a PCB concentration of 9 ppm.

A puzzling fact for biota in the Baltic Sea is that levels of DDT and PCB have decreased since 1970, as seen, for example, in herring (Olsson and Reutergård 1986). This decrease may also have occurred in salmon, although no systematic studies have been carried out for this species. Consequently, any increased fry mortality should have been observed in the 1960s when concentrations of persistent pollutants were supposed to be high. However, there may be an explanation for the outbreak of M74 since 1974. In recent decades the Baltic Sea has been subjected to eutrophication (e.g., Wulff et al. 1986). The eutrophication has favored herring, and their populations have increased. Herring contain large amounts of fat and accumulate lipophilic pollutants to high levels. Salmon exploit this food resource to a greater extent now than earlier, and consume greater amounts of persistent pollutants than when they were eating other food, such as other fish species and benthos. The pollutant content of prey is one of the factors governing uptake in predators (Borgmann and Whittle 1992; Madenjian et al. 1993). Consequently, as levels of persistent pollutants have increased, the reproductive ability of salmon has decreased. At the same time, extended foraging migrations of salmon have decreased as the herring now is present nearer the coast, and salmon growth rate and fat content have increased, as well as pollutant accumulation in the body and in the roe. The reproductive failure after 1974 caused by pollutants but at a time when environmental pollution was decreasing may therefore have an ecological explanation.

Acknowledgments

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Comparison of reliability of catch-at-age analyses using auxiliary survey estimates of relative or absolute recruitment

Joseph E. Hightower

Abstract: I used simulations based on a trawl fishery for widow rockfish (*Sebastes entomelas*) to compare the reliability of catch-at-age analyses using auxiliary survey estimates of either relative or absolute recruitment. Survey estimates of recruitment were assumed to have a coefficient of variation (CV) of either 0, 25, or 50%. Catch-at-age analyses were done in years 8–20 of a 20-year management horizon, on the basis of 7–19 years of catch-at-age and survey data. Errors in estimated stock size were autocorrelated and were often large in years 8–12 but decreased considerably once about 12–15 years of data were available. For survey CVs of 25–50%, a precise (±20%) estimate of stock size was about twice as likely using survey estimates of absolute abundance than of relative abundance. Frequently, similar results were obtained for imprecise, absolute abundance surveys (CV = 50%) and more precise, relative abundance surveys (CV = 25%). Because of larger errors in estimating abundance and setting catch quotas, population trajectories varied over a wider range when a relative abundance survey was used. In one case (target fishing mortality rate of 0.25, survey CV of 25%), linear trends in biomass were estimated reliably using either relative or absolute abundance data.

Résumé : J'ai utilisé des simulations basées sur une pêche au chalut de la veuve (*Sebastes entomelas*) pour comparer la fiabilité des analyses des captures selon l'âge à l'aide d'estimations fournies par des relevés auxiliaires portant sur le recrutement relatif ou absolu. On posait que ces estimations du recrutement avaient un coefficient de variation (CV) de 0, 25 ou 50%. Les analyses des captures selon l'âge ont été effectuées annuellement à compter de la 8^{e} année d'un horizon de gestion de 20 ans, à partir des données de la 7^{e} à la 19^{e} année sur les captures selon l'âge et de celles fournies par les relevés. Les erreurs dans la taille estimée du stock étaient autocorrélées; elles étaient souvent grandes dans la période de la 8^{e} à la 12^{e} année, mais elles baissaient considérablement à partir du moment où on disposait de 12 à 15 années de données. À des CV de relevés se chiffrant à 25–50%, une estimation précise (±20%) de la taille du stock était environ deux fois plus probable lorsqu'on mesurait l'abondance absolue plutôt que l'abondance relative. Il arrivait fréquemment que des résultats similaires soient obtenus pour des relevés imprécis de l'abondance absolue (CV = 50%) et pour des relevés plus précis de l'abondance relative (CV = 25%). À cause du plus grand degré d'erreur dans l'estimation de l'abondance et dans l'établissement des quotas de capture, les projections démographiques variaient davantage quand on se fiait à un relevé de l'abondance relative. Dans un cas (taux cible de mortalité par pêche de 0,25, CV du relevé de 25%), on a pu estimer de façon fiable les projections linéaires relatives à la biomasse à l'aide de données sur l'abondance, qu'elle soit relative ou absolue.
[Traduit par la Rédaction]

Introduction

Analyses of catch-at-age data (Murphy 1965; Doubleday 1976; Deriso et al. 1985; Gavaris 1988; Methot 1990) form the basis for management of many exploited fish stocks. To obtain reliable estimates from a catch-at-age analysis, auxiliary data such as survey catches or fishing effort are typically needed to constrain the analysis (Deriso et al. 1985). Survey catches can provide estimates of either relative or absolute abundance, depending on the type of gear used and the species of interest. Relative abundance data tend to be easier and less costly to obtain, but may be much less effective than estimates of absolute abundance in constraining a catch-at-age analysis. In an assessment model for walleye pollock (*Theragra chalcogramma*), Kimura (1989) found that three survey estimates of abundance near the end of the assessment interval were an adequate constraint only if assumed to measure absolute abundance.

The objective of this study was to compare the reliability of abundance estimates from a catch-at-age analysis, given annual survey estimates of either relative or absolute abundance of recruits. I made this comparison using a model of the fishery and assessment process for widow rockfish (*Sebastes entomelas*), a long-lived species exploited by trawling off the coasts of Washington, Oregon, and California. It is not my

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intent to provide an assessment for widow rockfish, but rather to provide information about the usefulness of recruitment surveys that measure either relative or absolute abundance. That information may be helpful in allocating resources between surveys, and in setting research priorities. The specific results from this study regarding the reliability of assessment results should be generally applicable to long-lived species for which auxiliary survey estimates of recruitment are available.

Methods

I used a simulation model to represent the 20-year period beginning in 1980, when the fishery for widow rockfish began. The simulation included components representing widow rockfish population dynamics, operation of the fishery, and the following management activities: sampling the commercial landings to estimate catch at age, conducting a survey to estimate relative or absolute recruitment, conducting a stock assessment to estimate abundance, and setting the catch quota. Details of the different simulation components are provided in the following sections.

Simulation of the fishery

I used an age-structured model of the widow rockfish stock to simulate population change. For fish from the age at recruitment (5) through age 12,

$$N_{y+1,a+1} = N_{y,a} \exp(-M - F_{y,a})$$

where $N_{y,a}$ represents the number of age-a fish in year y, M is the assumed instantaneous natural mortality rate (0.15), and $F_{y,a}$ is the instantaneous fishing mortality rate for age-a fish in year y. Fish aged 13 years and older were accumulated in a single bin ($N_{y,13+}$):

$$N_{y+1,13+} = N_{y,12} \exp(-M - F_{y,12}) + N_{y,13+} \exp(-M - F_{y,13+})$$

Catch in numbers was obtained from the standard catch equation:

$$C_{y,a} = N_{y,a} F_{y,a} (1 - \exp(-Z_{y,a}))/Z_{y,a}$$

where $Z_{y,a}$ represents total instantaneous mortality ($M + F_{y,a}$) for age-a fish in year y. The catch in biomass was obtained as $\Sigma C_{y,a} w_a$ where w_a is the mean weight of age-a fish (assumed constant over time). Stock biomass was estimated similarly using $N_{y,a}$ and w_a. I assumed that fishing mortality in year y was a product of a year-specific rate (F_y) and an age-specific selectivity (s_a) that was constant over time:

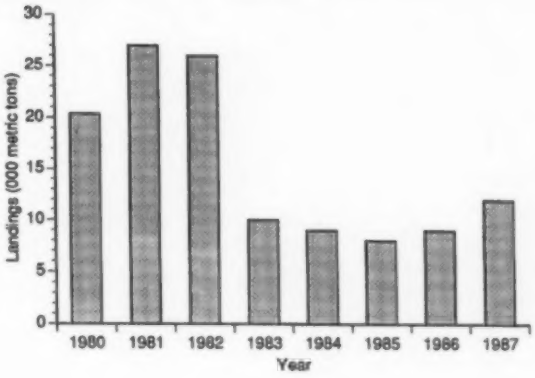
$$F_{y,a} = F_y s_a$$

Recruitment in year y ($N_{y,5}$) was described by a Beverton–Holt stock-recruitment curve of the form proposed by Kimura (1988):

$$N_{y,5} = R_\infty \frac{(S_{y-5}/S_\infty)}{(1 - A(1 - S_{y-5}/S_\infty))} \exp(\varepsilon_y)$$

where R_∞ was the recruitment level associated with the virgin spawning stock S_∞, S_{y-5} was the spawning stock in year y–5 that produced R_y, A was the level of density dependence, and $\exp(\varepsilon_y)$ was a lognormal error term used to introduce random variability in recruitment. To parameterize the model, R_∞ was set equal to the back-transformed mean of the logarithm of historical recruitment values. The normal random variate ε_y was assumed to have mean of 0 and a variance (0.507) based on the log-scale recruitment values. The product $R_\infty \exp(\varepsilon_y)$ would have the same distribution as the historical recruitment values (Law and Kelton 1982). Estimates of A were not available; consequently, I assumed arbitrarily that recruitment decreased by 10% when spawning stock decreased by 50%. That assumption was used in an earlier simulation study (Hightower 1990) to introduce a degree of density dependence into a model of the widow rockfish stock. I assumed that R_2–R_5 were produced by a stock equal in size to S_1 because fishing mortality was negligible prior to 1980, when the fishery began.

Fig. 1. Landings from the Washington–Oregon–California widow rockfish fishery for 1980–1987.



The initial number-at-age vector, selectivity pattern, and mean and variance for recruitment were estimates obtained from the 1988 assessment (Lenarz and Hightower 1988). To mimic the fishing-down process that occurred in the widow rockfish fishery (Lenarz and Hightower 1988), the annual catch in the simulated fishery in years 1–8 equalled the observed 1980–1987 landings (Fig. 1). This was done to better understand the reliability of the actual widow rockfish assessment, which was an objective unrelated to this paper. Because all replicate simulations shared the initial number-at-age vector and catch in years 1–8, F values in years 1–8 differed only as a result of random variation in recruitment. In subsequent years, the quota (which equalled the catch within the simulations) was based on a target fishing mortality rate (F = 0.05, 0.15, or 0.25) and the estimate of current abundance from the simulated assessment. The fishing mortality rate in years 9–20 differed from the target rate because of errors in estimating abundance.

The port sampling and survey sampling processes were simulated to produce the observed catch-at-age data and survey estimates of recruitment. As in earlier studies (Pope and Gray 1983; Kimura 1989), the simulated catch-at-age data were assumed to have lognormally distributed errors. I assumed a coefficient of variation (CV) of 10%, on the basis of estimates by Parker and MacCall (1990) for widow rockfish beyond the age of full recruitment. The simulated survey abundance estimates were assumed to have lognormally distributed errors and coefficients of variation (CV) of either 0, 25, or

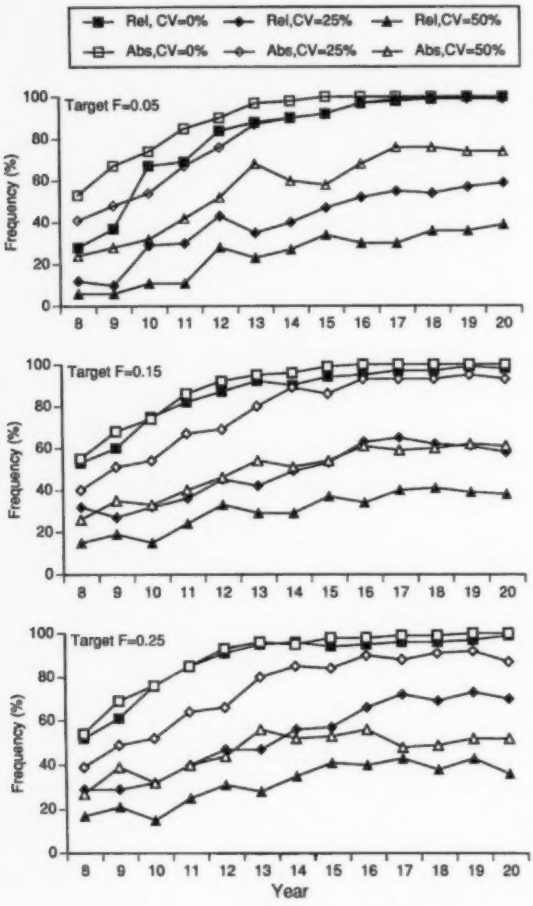


Fig. 2. Percentage of trials for which the estimate of current biomass (e.g., year-7 biomass for the assessment in year 8) was within 20% of the true value. Treatments represent target F values of 0.05, 0.15, or 0.25 and survey CVs of 0, 25, or 50% measuring either relative or absolute abundance.

50% (Rivard 1981; Pope and Gray 1983; Weinberg et al. 1984).

Stock assessment model

The Doubleday–Deriso catch-at-age model (Doubleday 1976; Deriso et al. 1985, 1989) was used in years 8–20 to estimate abundance of the simulated stock. The least-squares estimates of abundance, fishing mortality, selectivity, and (for relative abundance data) survey catchability were those that minimized

$$\sum_{y,a}(\log C_{y,a} - \log\hat{C}_{y,a})^2 + \lambda \sum_{y} (\log R_y - \log(\hat{q}\hat{R}_y))^2$$

where $\hat{C}_{y,a}$ was the predicted catch of age-a fish in year y, λ was the relative weight given the auxiliary survey data, R_y was the observed survey estimate of relative or absolute recruitment in year y, $\hat{q}$ was a parameter of proportionality, and $\hat{R}_y$ was the predicted number of age-5 fish (recruits) in year y. Following Kimura (1989), I used adjusted catches and survey values in the fitting process so that the predicted values would be unbiased on the original (back-transformed) scale. For those cases in which R_y was an estimate of the absolute number of recruits ($N_{y,5}$), $\hat{q}$ was set equal to 1.0. Marquardt's method was used to obtain least-squares estimates for the parameters (Marquardt 1963; Press et al. 1986).

Following the approach used in the computer program CAGEAN (Deriso et al. 1985; Quinn et al. 1990), the starting values for the least-squares fit were obtained through an approximate cohort analysis (Pope 1972). The results of the catch-at-age analysis were used to project population size 1 year into the future (N_{y+1}) and to calculate the quota for that year. Following the approach currently used to project population size for widow rockfish, recruitment in year $y + 1$ was assumed to be equal to the estimated historical average. The process was repeated for years 8–20 of a 20-year planning horizon.

I assumed that the relative precision of the catch data versus the survey data was not known. Variance estimates frequently are not available for catch-at-age and survey data. Even when available, the variances may not reflect all sources of variability (e.g., if ageing error or the effect of using an age–length key was ignored, or if a survey measuring the abundance of prerecruits was used as an index of numbers subsequently recruiting). Preliminary results in this study were similar using λ values of 0.5 and 1.0; consequently, I used an λ value of 0.5 for all cases. Deriso et al. (1985) used auxiliary estimates of fishing effort in an assessment for Pacific halibut (*Hippoglossus stenolepis*) and observed that results were similar for λ values between 0.1 and 1.0. It should be noted that when the variances are known, λ should equal

$$\mathrm{var}(\log C_{y,a}) / \mathrm{var}(\log R_y)$$

(Deriso et al. 1985, 1989; Kimura 1989). An approximate estimate based on the delta method is

$$(\mathrm{CV}(C_{y,a}) / \mathrm{CV}(R_y))^2$$

(Kimura 1989). For a catch CV of 10% and survey CVs of 0, 25, and 50%, the corresponding λ values would be ∞, 0.16, and 0.04, respectively.

I assumed that the natural mortality rate and selectivity at age 10, the age at full recruitment, were known without error. Fixing selectivity at 1.0 for at least one age is a necessary constraint in a catch-at-age analysis (Deriso et al. 1985; Kimura 1989). Separate selectivities were estimated for all other ages, and those estimates were sometimes greater than 1.0 because of errors in estimating the age at full recruitment. In preliminary runs, substantial errors sometimes resulted from extremely low selectivity estimates for the oldest age group (13+), with a correspondingly large overestimate of the number of age-13+ fish. Consequently, I constrained selectivity to the range 0.01 to 3.00 to rule out extreme solutions that an assessment scientist would be unlikely to accept.

Experimental design

I evaluated 18 treatment combinations: 2 survey types (relative, absolute), 3 levels of survey CV (0, 25, 50%), and 3 target F values for years 9–20 (0.05, 0.15, and 0.25). One hundred replicate 20-year runs were made for each treatment

Fig. 3. Percentage of trials for which the estimate of current biomass (e.g., year-7 biomass for the assessment in year 8) was within 50% of the true value. Treatments represent target F values of 0.05, 0.15, or 0.25 and survey CVs of 0, 25, or 50% measuring either relative or absolute abundance.

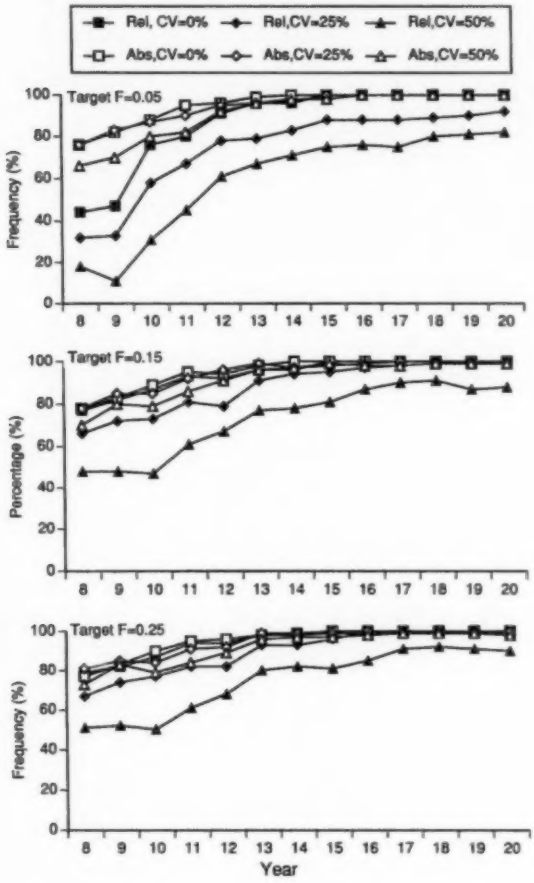
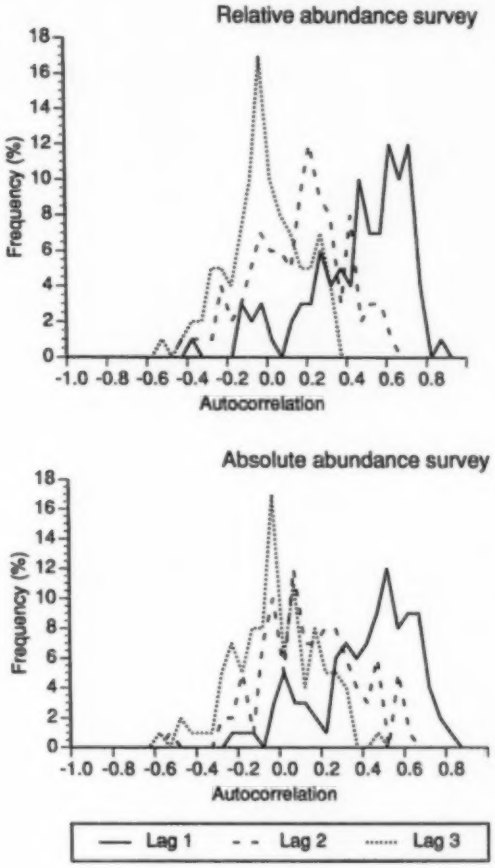


Fig. 4. Distribution of estimates of the autocorrelation for percent relative error ((true–estimate)/true × 100) for current biomass, as illustrated for a target F value of 0.25 and a survey CV of 25%. The upper and lower panels represent assessments using survey estimates of relative and absolute abundance of recruits.



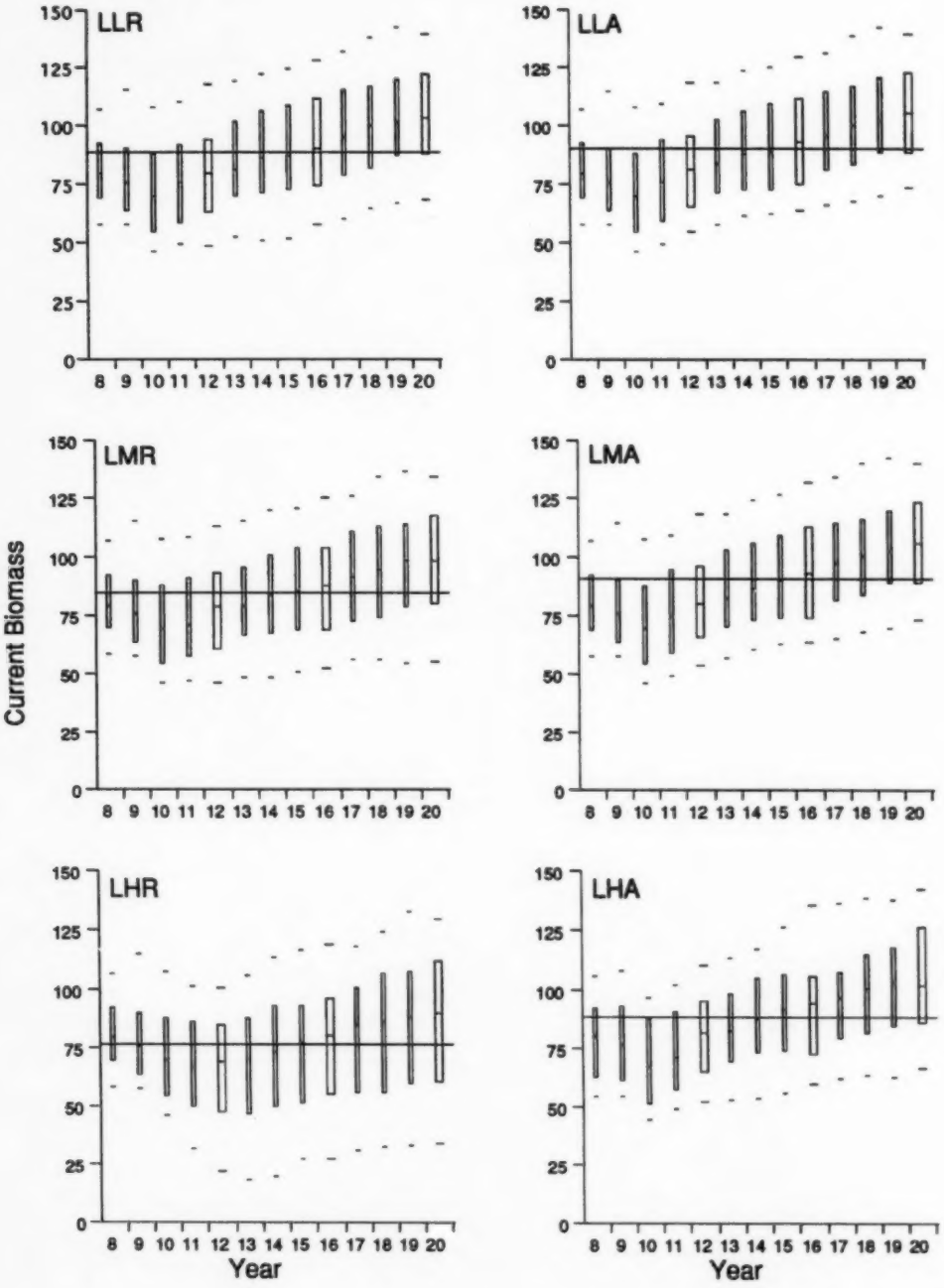
combination except that, because of damage to a data file, only 50 replicates were usable for one treatment (target $F = 0.05$, survey CV = 50%, absolute abundance).

Along with many other species, widow rockfish are managed using quotas, which are based on the most recent estimate of abundance and assumed levels of recruitment and fishing mortality. To evaluate the reliability of that process, I examined errors in estimated stock biomass in the final year of each assessment interval (termed current stock biomass), for example, biomass in year 7 for the assessment conducted in year 8. I determined the proportion of replicates producing current stock biomass estimates within 20 and 50% of the true values. I also determined whether errors in estimating current stock biomass were autocorrelated, and examined the effect of assessment errors by comparing population trajectories observed for each treatment combination. For one case (target fishing mortality rate of 0.25, survey CV of 25%), I examined the reliability of linear trends in estimated biomass, obtained from assessments using either relative or absolute abundance data. For each replicate assessment, I compared the slope of a line relating year and true biomass to the slope relating year and estimated biomass (for example, over years 1–7 for a year-8 assessment).

Results

Reliability of the simulated stock assessments was higher when using survey estimates of absolute, rather than relative, abundance, more precise survey data, a longer data series, and higher target F values. A precise estimate of current stock size (±20%) was about twice as likely when using a survey measuring absolute abundance, than when using a relative abundance survey, unless the survey CV was 0 (Fig. 2). Stock size estimates were about equally reliable for an imprecise, absolute abundance survey (CV 50%) and a more precise, relative abundance survey (CV 25%). Estimates were generally within

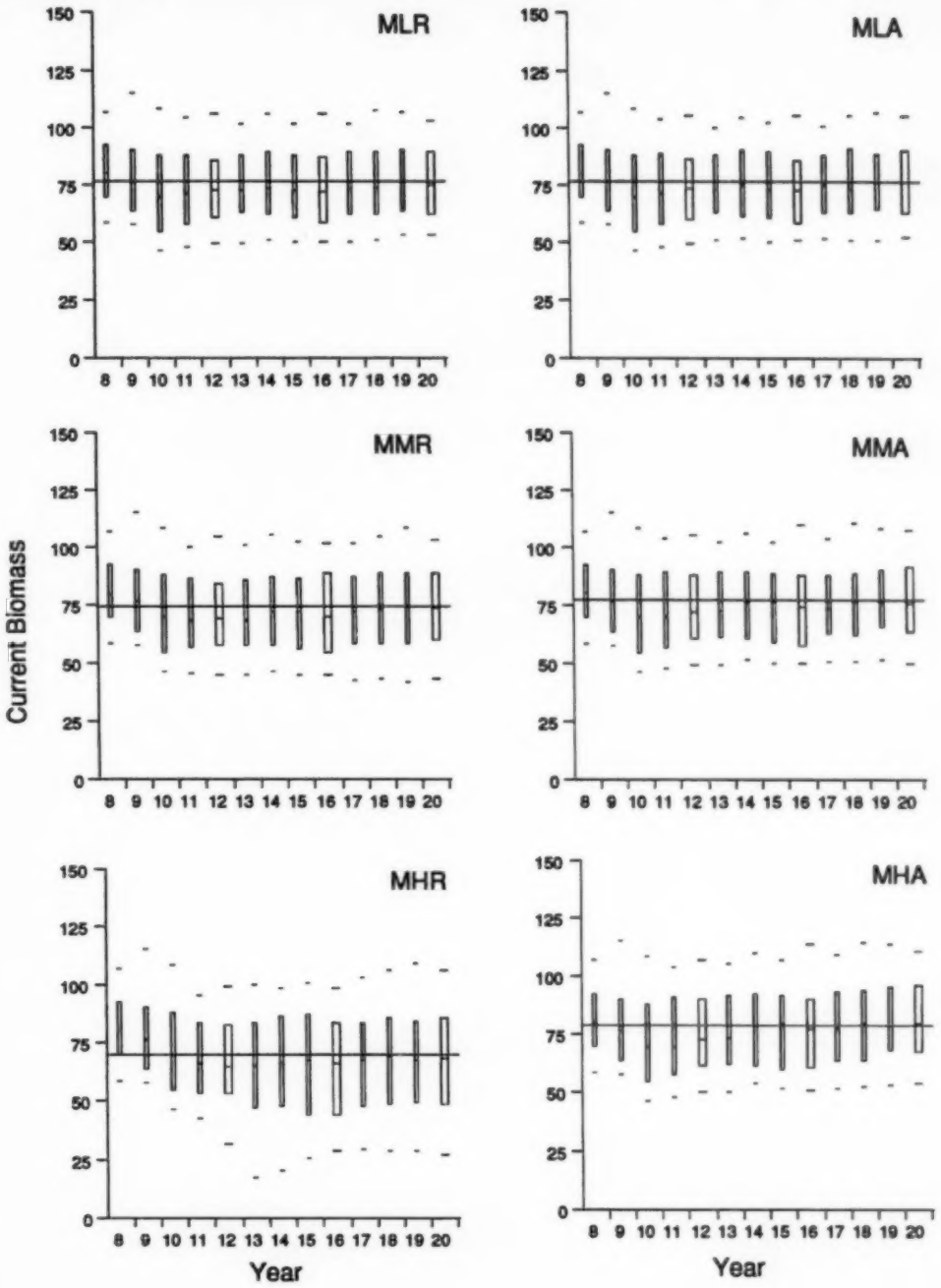
Fig. 5. Quantiles (90, 75, 50, 25, 10%) for simulated current biomass in years 8–20. Each treatment is represented by a three-letter code, representing target F value (L, 0.05; M, 0.15; H, 0.25), survey CV (L, 0%; M, 25%; H, 50%), and survey type (R, relative abundance; A, absolute abundance). The horizontal line represents mean biomass over all years.



50% of the true value except when the target F was 0.05 or when an imprecise relative abundance survey was used (Fig. 3). The difference in performance between a relative and absolute abundance survey tended to decrease somewhat as the target F or the length of the data series increased (Fig. 3).

Errors in current stock biomass estimates from consecutive

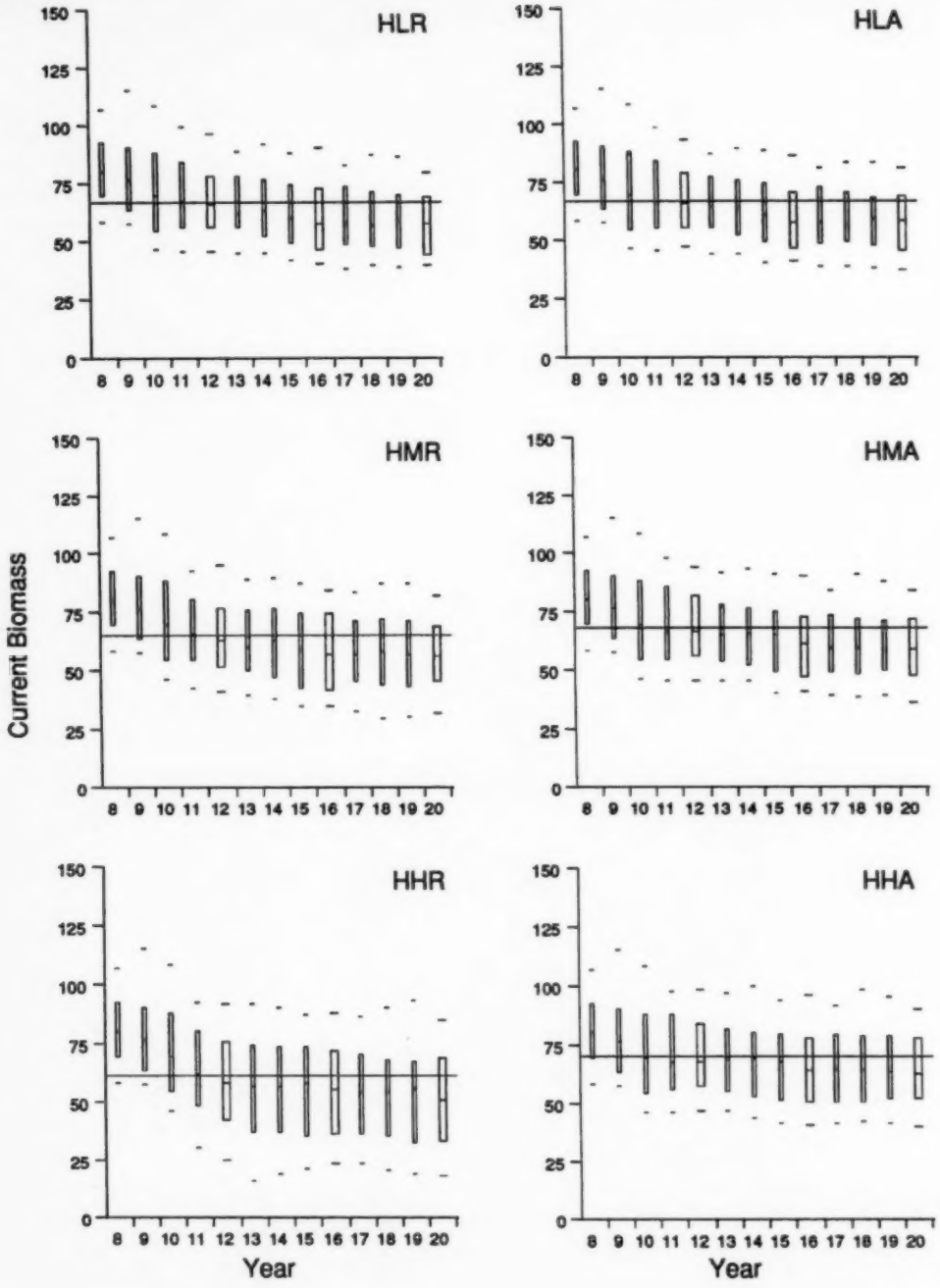
Fig. 5 (*continued*).



assessments tended to be autocorrelated, even though a separate assessment was done each year (including producing new starting values for the least-squares fitting routine). Results for a target F of 0.25 and survey CV of 25% (Fig. 4) illustrate the pattern that the correlation is substantial for a 1-year lag but decreases to about zero within 3 years. The distribution of autocorrelation estimates was similar for relative and absolute abundance data.

Errors in estimating abundance moderately affected the population trajectories observed for each treatment combination (Fig. 5). Stock size varied more widely and reached low levels more commonly when assessments were based on

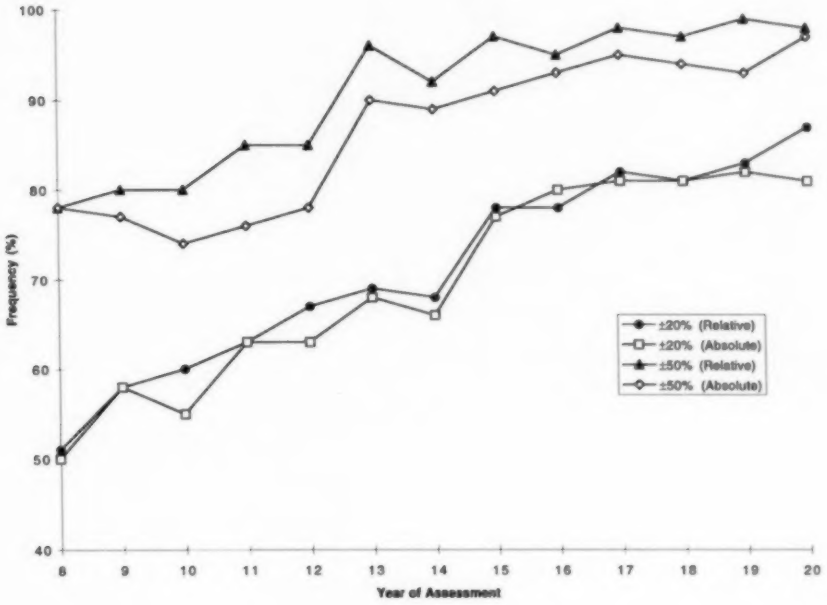
Fig. 5 (*concluded*).



relative abundance data. When assessments were based on imprecise (CV 50%) relative abundance data, stock size sometimes dropped to low levels in years 12–15 because of errors in setting the annual quotas.

Detailed results for one case (target fishing mortality rate of 0.25, survey CV of 25%) indicated that linear trends in biomass could be estimated about equally well using either relative or absolute abundance data (Fig. 6). The reliability of the estimated trend increased moderately for both survey types as the length of the assessment period increased (Fig. 7).

Fig. 6. Percent frequency of estimates of the linear trend in estimated biomass over time that were within 20 and 50% of the trend for actual biomass, for a target F value of 0.25 and a survey CV of 25%.



Discussion

From a management perspective, the results of this and earlier simulation studies (Pope and Gray 1983; Kimura 1989; Bence et al. 1993) demonstrate the difficulty of estimating current stock size reliably when precise survey data are unavailable. For relative abundance surveys or imprecise absolute abundance surveys, there was a low probability of being within 20% of the true biomass level. In addition, assessment errors were highly autocorrelated, which would increase the likelihood of substantially under- or over-fishing. Unfortunately, some degree of autocorrelation seems unavoidable, given that the data set being analyzed changes only slightly from year to year.

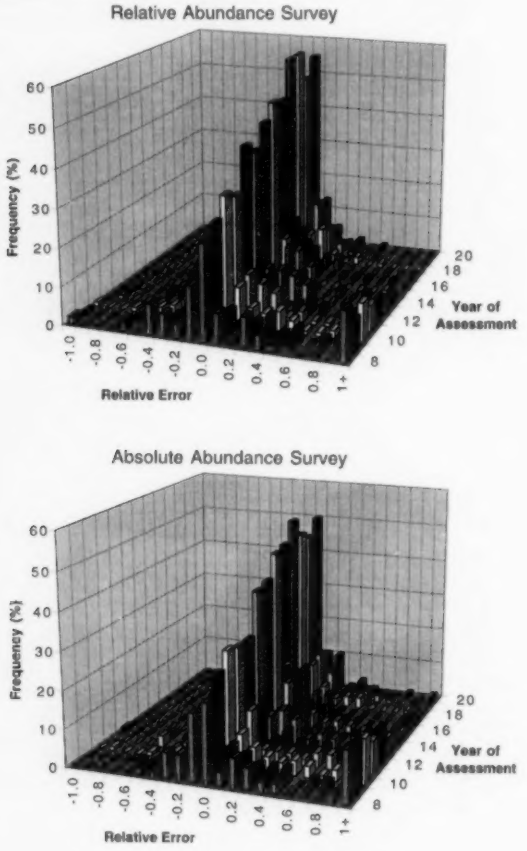
Compared with these simulated assessments, better results might be obtained by a knowledgeable analyst, using judgement or information not included in the assessment to reject unreasonable solutions. However, estimates of recent stock size made by experienced analysts have varied widely in assessments done in subsequent years (Rivard 1981). In addition, the simulated assessments do not take into account annual variability in selectivity or survey catchability, or uncertainty about natural mortality, all of which would further degrade the reliability of an assessment (see Restrepo et al. 1992).

The lower reliability of assessments using survey estimates of relative abundance seems reasonable because fishery catch-at-age data already contain much information on relative year-class strength (Ricker 1975; Methot 1990; Hilborn and Walters 1992; Bence et al. 1993). The critical questions for a fishery manager are whether absolute abundance can even be estimated through a survey and whether the additional cost and effort are justified by the gain in precision of the assessment. Another issue is the added risk of assuming that a survey measures absolute abundance. For example, a swept-area trawl survey may underestimate absolute abundance because of trawl avoidance, or possibly overestimate abundance owing to herding by the warps into the path of the trawl (Alverson 1971; Main and Sangster 1981; Laevastu and Favorite 1988). Hydroacoustic equipment is also commonly used to estimate absolute abundance, but the resulting estimates may be biased because of errors in estimating target strength (i.e., converting echoes into biomass) or because fish close to the bottom or surface cannot be surveyed adequately (Gulland 1975; Thorne 1983). One approach for addressing this concern is to assume that the survey is measuring relative abundance, then use Monte Carlo simulation (Restrepo et al. 1992) to test whether catchability is significantly different from 1. Given the uncertainty present in most assessments, however, a significant difference would be unlikely unless q was very different from 1.

A survey to measure absolute abundance will not be feasible in many instances. Thus, it is encouraging to note that average population trajectories were similar for assessments using relative and absolute abundance data, even though stock size reached lower levels more often when imprecise relative abundance data were used (Fig. 5). In addition, the difference in reliability of assessments based on relative and absolute abundance surveys diminished somewhat as the length of the data series increased. This presumably reflects improved calibration of the relative abundance data (i.e., a better estimate of q).

For a case in which biomass was declining over time (target $F = 0.25$), it is also encouraging that linear trends in historical

Fig. 7. Distribution of estimates of relative error ((true – estimate)/true) for the linear trend over time in estimated biomass compared with true biomass, for a target F value of 0.25 and a survey CV of 25%. The upper and lower panels represent assessments using survey estimates of relative and absolute abundance of recruits.



biomass were estimated equally reliably when using relative or absolute abundance data. Although trends in biomass cannot be used directly in quota management, they may provide a reliable indicator that F is too low or too high. For these simulated assessments, the probability of estimating the trend within ±50% was high even for an analysis based on only 8–10 years of data. The observation that trends in biomass are estimated more reliably than absolute biomass has also been made for biomass-based models (Prager 1994).

One question that merits further work is whether annual surveys are necessary. In this study, simulated surveys were conducted annually and estimates of current biomass were increasingly reliable as the length of the data series increased. Kimura (1990) used simulation to show that precise survey estimates of absolute abundance at the beginning and end of a time series adequately constrained a catch-at-age analysis, whereas a survey conducted only at the end of the time series did not. Results from this study suggest that a survey of absolute abundance could be less precise or perhaps conducted for fewer years than a relative abundance survey and still provide comparable assessment results.

Acknowledgments

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Seasonal current holding performance of juvenile Atlantic salmon in relation to temperature and smolting

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Abstract: The ability of juvenile Atlantic salmon to hold station against a water current was tested in relation to season, environmental temperature, and developmental strategy. The finding that holding velocities, relative to body length, were similar in the faster growing, earlier smolting fish (upper modal group) and the fish remaining in freshwater for a further year (lower modal group) suggests that there was no difference in relative performance or habitat availability. Seasonal changes in performance were generally related to water temperature, with the decrease in holding ability becoming more pronounced at low temperatures. The relative critical holding velocity of smolting fish was lower over the period of seaward migration than in previous months. However, within this period, critical holding velocity increased with degree of silvering. The implications of these results for the ecology and life-history strategy of juvenile salmon are discussed.

Résumé : L'aptitude des saumons atlantiques juvéniles à se maintenir en station contre le courant a été examinée en fonction de la saison, de la température ambiante et de la stratégie de développement. La similarité des vitesses de l'eau, relativement à la longueur du corps, chez les poissons à croissance rapide et à smoltification précoce (groupe modal supérieur) et chez ceux qui restent en eau douce une année de plus (groupe modal inférieur) permet de penser qu'il n'y a pas de différence dans la performance relative ni dans la disponibilité de l'habitat. Les changements saisonniers de la performance étaient en général reliés à la température de l'eau, mais la baisse de l'aptitude à maintenir la station était plus prononcée à basse température. La vitesse relative critique pour les poissons en smoltification était plus faible pendant la période de dévalaison par rapport aux mois précédents. Toutefois, pendant cette période, la vitesse critique augmentait avec le degré d'argenture. Nous analysons les incidences de ces résultats sur l'écologie et la stratégie de développement biologique des jeunes saumons.
[Traduit par la Rédaction]

Introduction

During the summer months juvenile Atlantic salmon (*Salmo salar* L.) tend to hold station against the current at a chosen focal point within a defended territory (Kalleberg 1958; Keenleyside and Yamamoto 1962). Most inhabit riffle-type habitats in areas of moderate to high velocity, and are usually in ventral contact with a home stone (Rimmer et al. 1983). They achieve this by using their pectoral fins as hydrofoils, thus providing negative lift (Wankowski 1981; Arnold et al. 1991). This gives them the opportunity to dart into adjacent areas of higher water velocity (with consequent higher rates of drifting prey) to capture particles of food (Wankowski and Thorpe 1979), so allowing them to minimize energy expenditure and maximize profits (Fausch 1984).

During the autumn there appears to be a sudden decrease in the numbers of parr visible in feeding areas of streams and this disappearance is related to temperature (Allen 1940; Stuart 1957; Bjornn 1971; Bustard and Narver 1975; Gibson 1978; Gardiner and Geddes 1980; Rimmer et al. 1983; Cunjak and Power 1986). Rimmer et al. (1983, 1984) and Heggenes and Saltveit (1990) showed that the fish do not move away from this area of the stream, but move into interstitial spaces in the cobble–boulder substrate.

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This sheltering behaviour may be important in keeping the energy expenditure of these fish to a minimum, because (being ectothermic) general performance is likely to be impaired at low temperatures. However, feeding does occur over the winter (Jones 1959; Riddell and Leggett 1981; Cunjak 1988), so some fish must emerge into the water column.

Higgins and Talbot (1985) and Metcalfe et al. (1986, 1988) showed in laboratory experiments differences in food intake over autumn and winter that lead to the bimodal distribution of size in a single sibling population of juvenile salmon and this phenomenon has since been demonstrated in the wild (Baglinière and Maisse 1985; Heggenes and Metcalfe 1991; Nicieza et al. 1991). The larger fish (upper modal group, UMG) smolt the following spring and the smaller fish (lower modal group, LMG) remain in freshwater for at least another year (Thorpe 1977). Throughout the winter period (from October to March), the UMG fish maintain a significantly higher daily food intake at any given temperature than the LMG fish (Higgins and Talbot 1985). This divergence in food intake is probably due to overwintering LMG fish displaying a greater degree of sheltering behaviour (F.A. Huntingford and J.E. Thorpe, personal observation) in combination with a lower rate of prey capture than UMG fish (Metcalfe et al. 1986, 1988), which in turn may have led to a difference between the two modes of fish in their ability to hold station in a current.

Rimmer et al. (1985) showed that the holding performance of juvenile Atlantic salmon generally varied with seasonal temperature, but more specifically fell dramatically at temperatures below 8°C, roughly equivalent to the temperature at which the parr move into interstitial spaces. This work was carried out on groups of parr of similar size and therefore did not address the question of differences in holding ability between UMG and LMG fish. A spring decrease in swimming ability has been recorded in smolting Atlantic salmon (Thorpe and Morgan 1978) and in smolting coho salmon (*Oncorhynchus kisutch*) (Smith 1982). Therefore, it would also be of interest to continue measuring critical holding velocity (CHV) into the period of seaward migration to see if the ability to oppose current changes over this period of time.

The purpose of the present study was to investigate the relationship between CHV in juvenile Atlantic salmon and (*i*) environmental temperature, (*ii*) life-history strategy, (*iii*) fish size and development, and (*iv*) seaward migration.

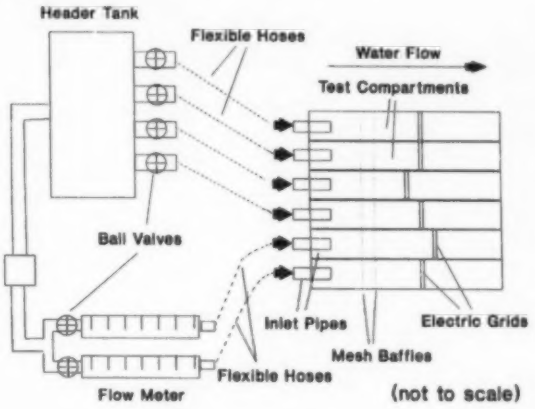
Materials and methods

Fish

The fish used were sibling offspring from sea-run Atlantic salmon raised initially at the Scottish Office Agriculture and Fisheries Department (SOAFD) smolt-rearing station at Almondbank, Perthshire, and subsequently at the SOAFD Freshwater Fisheries Laboratory in Pitlochry. In both places the fish were held in radial flow tanks (Thorpe and Wankowski 1979) under natural photoperiod and temperature and were fed dry hatchery pellets to excess.

The salmon used in the experiment were 0+ UMG and 0+ LMG fish (i.e., during the 1st year in freshwater), and 1+ LMG fish (i.e., during the 2nd and final year in freshwater). Before each test period each fish in the 0+ experimental population was weighed (to 0.1 g) and measured (fork length, to 1 mm) to allow classification into UMG or LMG (Cassie 1954; Thorpe 1977). The ranges of mean fork lengths of the fish over the experimental period were 72.7–123.4 mm, 57.0–79.4 mm, and 93.9–124.7 mm for 0+ UMG, 0+ LMG, and 1+ LMG fish, respectively. Fish with fork lengths close to the mean fork lengths of the respective modal groups were selected for use in the experimental procedures.

Fig. 1. Diagram of the experimental tank (total length 1.08 m) showing that low-velocity acclimatization water flow can be exchanged for adjustable experimental flow by switching the position of flexible hoses, thus avoiding disturbance to experimental fish.



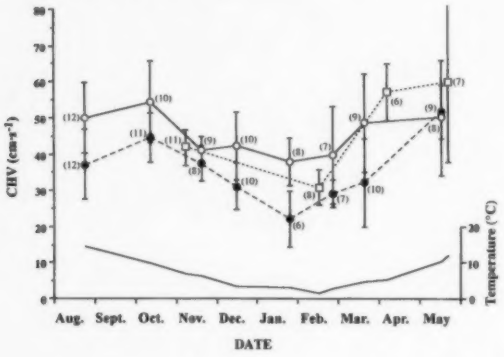
Equipment

To increase the time allowed for habituation of the test fish whilst obtaining sufficient measurements within a suitable time period, a tank with six raceways was built (Fig. 1). The raceways each had a trapezoidal cross section with the ventral surface wide enough for the largest parr to fully extend their pectoral fins. Each raceway had its own water inlet pipe separated from the test compartment by two plastic meshes to reduce turbulence.

The fish were forced to maintain position in the test compartment by an incomplete metal grid placed downstream, which was connected to the anode and cathode of a DC electrical converter. When a fish touched this grid it closed the circuit and was subjected to an electrical potential of 2 V. This was sufficient to keep the fish away from the grid without causing undue shock. The front part of the test compartment was lined with black insulating tape to encourage fish to maintain position in the compartment, and to help reduce any stress during habituation. Rimmer et al. (1985) reported that smooth plastic may reduce the measured performance of juvenile Atlantic salmon in velocity tests because they hold station on the substrate. To counteract this the bottom of each test compartment was covered in a layer of silicon sealant that adhered to the plastic and provided the fish with a slightly rougher substrate and thus potentially more grip.

The experimental water supply was introduced through one of two lines, each with taps below a graduated cylinder giving a readout in litres per minute. The water velocity in the test

Fig. 2. Seasonal changes in mean absolute critical holding velocity ($cm \cdot s^{-1}$) of 0+ UMG (open circles), 0+ LMG (solid circles), and 1+ LMG (open squares) fish, and water temperature (solid line). Vertical bars represent 1 standard deviation from the mean.



compartment was calibrated with this readout by using a Kent-Lea low-speed Miniflo probe (type 265-3) attached to a Nixon Streamflo 422 velocity meter (Nixon Instrumentation, Cheltenham, U.K.). These measurements were taken at the front of the test compartment (Fig. 1) to measure the focal point velocities experienced by the experimental fish.

The output from each of these lines could be attached to the inlet tube of any raceway, as could the other four lines coming from the header tank. This allowed four fish to be acclimated to the raceways while two others were being tested, and eliminated the need to move fish to test areas immediately prior to testing.

Test procedure

Each fish was anaesthetized with benzocaine, measured (fork length), and weighed, then allowed to acclimate to the raceway for at least 24 h prior to being tested. During acclimation the water flow from the header tank was held below 1 body length/s. When the test started the adjustable experimental flow was attached to the water inlet at a velocity of 1 body length/s. The movable electric grids in the test compartments were positioned for each fish so that to avoid making contact the fish had to hold station and could not slip backwards to any significant degree. The fish were continually monitored throughout the experiment.

The critical water velocities that the fish could withstand were calculated by Brett's method (1964) of increasing water velocities by a standard amount every hour until the fish cannot maintain station. This is given by the equation $CHV = V_i + (t_i/t_{ii} \cdot V_{ii})$ where CHV is the critical holding velocity, V_i is the highest velocity maintained for the prescribed period, V_{ii} is the velocity increment, t_i is the time (min) the fish hold station at the final increment before collapse, and t_{ii} is the prescribed period at each velocity increment (60 min).

When the fish could not hold station any longer they collapsed flat against the electrified grid and were immediately removed. This index has been shown to provide an accurate estimate of the maximum sustained speed derived by the fixed velocity technique (Brett 1967).

Because the tests were carried out on parr of varying size, the increment was calculated to be 1 body length/s on the basis of the fork length of the fish. The results are referred to as critical holding (rather than swimming) velocities (Rimmer et al. 1985), as salmon held station on the substrate, rather than swimming. In practice, once the juvenile salmon were introduced into the test compartments they aligned themselves quickly towards the flow of water and on the ventral surface of the raceway with their pectoral fins extended, a position they held for the majority of the acclimation and test periods. As the velocity increased the fish could be seen to press lower on the substrate with occasional flicks of the tail if they started to slide backwards. Actual swimming behaviour was seen only at the highest water velocities when some of the fish would rise off the substrate and show a very brief burst of swimming (usually 1–5 s) before collapsing onto the electrified grid.

Sampling protocol

Experiments on 0+ fish were conducted monthly from August 1990 to May 1991 (with the omission of September and April) and on 1+ fish during November 1990 and February, April, and May 1991. All experiments were carried out under natural temperatures and photoperiods. Sample sizes varied between 6 and 12 fish from each modal group per month owing to escapes and predation by feral cats.

During the sampling period in May, the experimental fish were photographed immediately prior to being introduced into the experimental tank. This allowed the extent of silvering in smolting fish to be graded using the following scale: 1, distinct parr marks, no silvering; 2, slight loss of parr marks accompanied by slight silvering; 3, distinct overall silvering, parr marks faint or nearly absent, but with some pigmented areas; 4, complete overall silvering.

Results

General seasonal trends

The absolute critical holding velocity (i.e., water velocity measured as centimetres per second) of all 0+ juvenile salmon varied over their 1st year in freshwater (Fig. 2, two-way ANOVA, $F_{[6,115]} = 8.283$, $P < 0.0001$), tending to decline with temperature over the autumn and winter months. Not surprisingly, the 0+ UMG fish (which were larger) were able to withstand higher water velocities than their smaller 0+ LMG counterparts (two-way ANOVA, $F_{[1,115]} = 50.284$, $P < 0.0001$). However, from February to May, while the absolute CHV of 0+ LMG increased steadily in line with an increase in water temperature of approximately 10°C, the absolute CHV of 0+ MG fish showed no significant change (one-way ANOVA, $F_{[2,21]} = 1.141$, $P = 0.339$). In Scotland (latitude 55–59°) the month of May tends to correspond to the final weeks of seaward migration in smolting fish. Overall, 1+ LMG fish (also in their final year prior to seaward migration) showed a seasonal pattern of absolute CHV similar to that of 0+ UMG fish.

When the CHV data were expressed in relative terms (i.e., body lengths/s) (Fig. 3) all fish showed the same ability to withstand water currents through the season (two-way ANOVAs, $F_{[1,115]} = 0.411$, $P = 0.565$; $F_{[1,44]} = 0.145$, $P = 0.170$, comparing 0+ UMG and 0+ LMG, and 0+ UMG and 1+ LMG fish, respectively), until May when the relative CHV

Table 1. Summary statistics of the multiple regressions relating relative CHV (body lengths/s) to temperature (°C) and fish fork length (mm) for three categories of fish.

	Regression coefficient	t	df	R^2	P
0+ UMG					
Temperature	0.284	4.56	64	0.0550	<0.0001
Fork length	0.010	0.50	64		0.62
0+ LMG					
Temperature	0.292	7.68	72	0.471	<0.0001
Fork length	0.047	1.99	72		0.0502
1+ LMG					
Temperature	0.390	6.89	24	0.657	<0.0001
Fork length	0.031	2.59	24		0.017

of 0+ UMG and 1+ LMG fish remained unchanged through a period of rising temperature while that of 0+ LMG fish increased. This resulted in the performance of smolting fish during the period of seaward migration being significantly poorer than that of nonsmolting fish (Kruskal–Wallis one-way ANOVA, $H = 12.10$, $P < 0.005$).

Critical holding velocity in relation to temperature

Regression analyses performed on relative CHV and the logarithm of relative CHV both showed highly significant effects with both temperature and the logarithm of temperature for 0+ UMG, 0+ LMG, and 1+ LMG fish. (Data collected from the last month when the fish were silvering were not used when examining relationships with temperature because of the obvious confounding effects.) However, 0+ LMG fish showed a slightly higher r^2 value when CHV was regressed against the logarithm of temperature. Because this was the largest of the data sets, it was decided to use the logarithmic transformation of temperature for all modal groups when comparing them.

There was no significant difference between periods of increasing and decreasing temperature in the slope or elevation of the CHV–log temperature regression lines within fish types (covariance analysis), thus allowing data to be plotted over the whole year. Comparisons between fish types showed similar CHV–temperature (logarithm) slopes for 0+ UMG and 0+ LMG fish, which were steeper than that for 1+ LMG fish ($t = 2.28$, df = 86, $P < 0.05$). Using the equations of these semilog plots, CHV was then plotted against temperature over the whole year for both of the 0+ modal groups (Fig. 4).

Effect of fish size

The effect of fish size on relative holding performance was determined for each life-history stage of fish by regressing relative performance against fork length and temperature over the whole year in a multiple regression. For 0+ UMG and 0+ LMG fish, temperature was the only variable that had a significant effect on CHV. However, in 1+ LMG salmon there was an independent effect of both temperature and fork length, with large fish at a given temperature having a higher relative CHV than small fish (Table 1).

Effect of degree of silvering

Figure 5 shows that as the amount of silvering increases (i.e., the external appearance of the fish become less like that of a parr and more like that of a smolt) in both 0+ UMG and 1+ LMG fish, the relative CHV increases (Spearman rank correlation $r_s = 0.779$, $N = 12$, $P < 0.02$). There was no correlation between degree of silvering and either water temperature, date, or fork length.

Fig. 3. Seasonal changes in mean relative critical holding velocity (body lengths/s) of 0+ UMG, 0+ LMG, and 1+ LMG fish, and temperature. See caption of Fig. 2 for explanation of symbols and vertical bars.

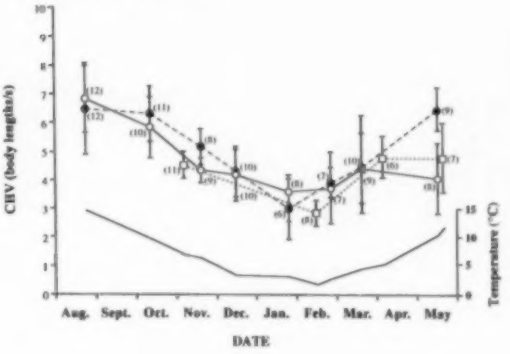
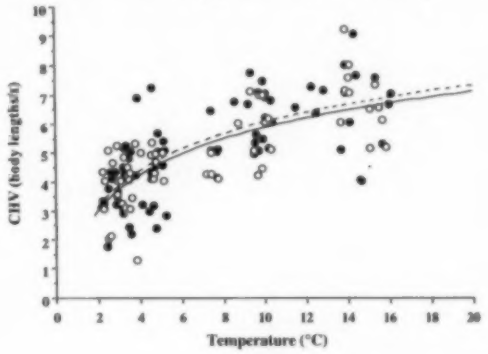


Fig. 4. Effect of seasonal temperatures on mean relative critical holding velocities of 0+ UMG (open circles) and 0+ LMG (solid circles) fish. The regression lines (0+ UMG: $y = 4.14 \log X + 1.74$, $r^2 = 0.746$, $n = 65$, $P < 0.00001$; 0+ LMG: $y = 4.24 \log X + 1.83$, $r^2 = 0.680$, $n = 73$, $P < 0.00001$) are derived from the semilog plots of CHV versus temperature.



Discussion

Studies of chosen snout velocities have shown slight differences between parr of different year-classes, with chosen velocities tending to increase with age and therefore size (e.g., Rimmer et al. 1984; DeGraaf and Bain 1986; Morantz et al. 1987; Gibson 1993). There is, however, a great deal of overlap between year-classes in these velocities and this must be even greater between modal groups of the same year-class. Indeed, the difference in absolute CHV between modal groups in the present study only varied between 4 cm·s^{-1} (November) and 17 cm·s^{-1} (January) (Fig. 2). The lowest mean absolute velocity was recorded for 0+ LMG fish in January (23 cm·s^{-1})

Fig. 5. The effect of silvering on mean relative critical holding velocity (body lengths/s) of 0+ UMG fish (open circles) and 1+ LMG fish (open squares) during the period of seaward migration (May).

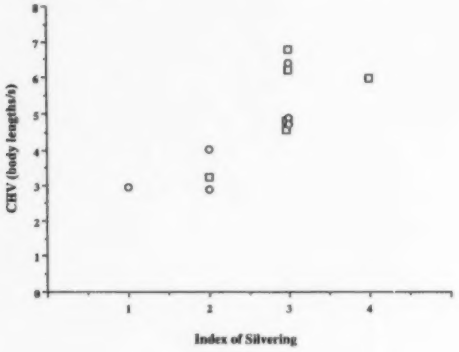
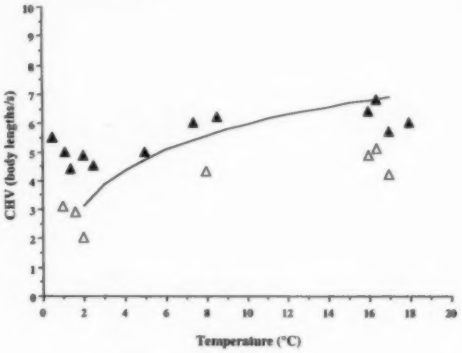


Fig. 6. A comparison of the effect of seasonal temperatures on the relative critical holding velocity (body lengths/s) of hatchery 0+ LMG fish (solid line) from the present study, and of wild (solid triangles) and hatchery (open triangles) fish from Rimmer et al. (1985).



and the highest was for 0+ UMG fish in October (55 $cm \cdot s^{-1}$), values that are generally comparable to those recorded by the above authors for juvenile salmon aged 0+ to 2+, and of a similar size to those in the present study. It should be noted that this is a comparison of velocities from parr forced to hold station with chosen summer focal point velocities; however, it still demonstrates that although members of the UMG (larger) have higher absolute critical holding velocities than those of the LMG (smaller), the differences would not be sufficient to cause differences in habitat availability, considering the range of chosen velocities reported within year-classes.

Another important aspect of the adoption of differing life-history strategies was examined by expressing critical holding velocities relative to body size. Prior to smolting, there was no difference in the relative performance of the upper and lower modal groups of parr (Fig. 3). Therefore, allowing for differences in body size, fish in the LMG are capable of working as efficiently as those in the UMG. In physiological terms, the life-history strategy adopted by the LMG fish can then be viewed as a developmental option, rather than as a strategy the fish have been forced to adopt because they are physiologically less capable than UMG fish.

This view is supported in the present study by the fact that there was an identical seasonal pattern of relative holding velocity for 0+ UMG and 1+ LMG fish. These were fish of different ages that had followed two different life-history strategies (evident in the bimodal split) and that would both smolt the following spring, but over their 1st year in freshwater had belonged to dominant and subordinate groups, respectively. This also demonstrates that the different behavioural traits exhibited by LMG parr (Huntingford et al. 1988), in particular seasonal anorexia (Metcalfe and Thorpe 1992) and subsequent food intake at maintenance ration level (Higgins and Talbot 1985), do not have long-term effects on the subsequent performance of these fish during the year prior to seaward migration.

This study has also demonstrated that all fish, regardless of life-history strategy, showed a decrease in CHV with declining temperature. Figure 6 shows the relative CHV values from nonsmolting wild and hatchery unimodal yearling Atlantic salmon over a whole year (from Rimmer et al. 1985) plotted against seasonal temperature and superimposed on the equivalent data for 0+ LMG fish from the present study. The results from the two studies are roughly comparable. Rimmer et al. (1985) suggested that their data showed a plateau in CHV values as temperature decreased from 15 to 8°C and a rapid decline in CHV from 8 to 2°C, followed by an increase in CHV values from 2 down to 0.5°C, whereas the CHV data from the present study showed more of a gradually sloping decline with temperature and no upturn in CHV values at low temperatures. The difference between the two sets of values from hatchery fish is probably due to those from the present study having been in contact with a rough substrate, thus allowing greater grip. Rimmer et al. (1985) noted that their use of a smooth Plexiglas substrate may have led to an underestimation of CHV values.

Gardiner and Geddes (1980) and Rimmer et al. (1985) postulated the existence of a critical threshold temperature, with sheltering behaviour triggered when water temperatures drop below this point in autumn. Rimmer et al. (1985) suggested that this threshold coincides with an abrupt decline in CHV owing either to an inability to adapt completely to low temperatures, or to the fact that it becomes more efficient to remain inactive at low temperatures (Gibson 1978). However, there appears to be geographical variation in the temperature at which this switch occurs. In the present study there was no abrupt decrease in CHV, but a gradual decline that became more severe as the temperature dropped below 6°C. This temperature is similar to that at which juvenile Atlantic salmon have been observed to take shelter in the British Isles (e.g., 7°C, Allen 1940; 5°C, Gardiner and Geddes 1980), whereas the drop in CHV and sheltering seems to occur at higher temperatures in eastern Canada (e.g., 9–10°C, Gibson 1978; Rimmer et al. 1985).

It has been suggested (Riddell and Leggett 1981) that in juvenile salmon living in high-velocity streams, longer pectoral fins are selected for, to reduce the energetic costs of maintaining position. However, Rimmer et al. (1985) acclimated similar-sized parr to different temperatures and showed their CHV to be the same as that of fish experiencing similar temperatures throughout the year, proving that temperature,

not variations in relative fin size (resulting in changes in body size with growth), has the greatest influence on CHV.

McCleave and Stred (1975) and Thorpe and Morgan (1978) showed that after failure to hold station against increasing water flows, smolts do not show the signs of stress and fatigue shown by parr in a similar situation. Thorpe and Morgan (1978) interpreted this to be either a behavioural refusal or an inability to undergo sustained swimming. The latter seems most likely, because in all of these tests (including the present study) fish were forced to hold station upstream of an electric grid. This interpretation agrees with the findings of Virtanen and Forsman (1987) that smolts of Atlantic salmon have a lower physiological capacity for swimming than parr, possibly because smolts have higher metabolic rates than parr (Higgins 1985; Maxime et al. 1989), which would reduce their capacity for sustained activity (Thorpe and Morgan 1978). However, it should be noted that sustained swimming is not a natural behaviour exhibited by parr.

The results showing that CHV increased with silvering seem to contradict this idea of lower relative holding or swimming performance in smolts than in freshwater residents. However, the association of degree of silvering with degree of smolting may not be a valid assumption. According to McMahon and Hartman (1988) silver colouring was not a reliable indicator of migratory behaviour in juvenile coho salmon (*Oncorhynchus kisutch* Walbaum) in Carnation Creek, Canada, where only 50% of the migrants entering the estuary were completely silvered. Kato (1972) has shown that silvering can be induced in rainbow trout (*Oncorhynchus mykiss*) by holding them in blue tanks, suggesting that silvering serves to camouflage them in a pelagic environment (Denton 1971; Kazakov and Koslov 1985).

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In situ target strengths of Atlantic cod (*Gadus morhua*) and Atlantic mackerel (*Scomber scombrus*) in the Northwest Atlantic

Allen Clay and Martin Castonguay

Abstract: The accurate determination of target strength (TS) is essential in the conversion of area backscattering to fish biomass. We present the first detailed in situ TS measurements of Atlantic cod (*Gadus morhua*) and Atlantic mackerel (*Scomber scombrus*) in the Northwest Atlantic. Mean total lengths of cod and mackerel were 52 and 38 cm, respectively. The mean daytime and nighttime in situ TS distributions (split-beam method) of cod were –35.5 (b_{20} = –69.8) and –35.2 decibels (dB) (b_{20} = –69.5), respectively. There was a depth stratification of cod TS at night, as mean in situ TS was –31.7 dB between 140 and 220 m and –35.8 dB between 220 and 300 m. We argue that the effect of depth on swim-bladder inflation is primarily responsible for the lower cod TS near the bottom than higher up in the water column. Analysis of the same TS data in split-beam or single-beam mode yielded similar distributions, showing the reliability of the single-beam method. The mean in situ TS (single-beam method) of mackerel was –56.4 dB (b_{20} = –88.0), with no day–night difference.

Résumé : La conversion de l'aire de rétrodiffusion acoustique en biomasse de poissons requiert que l'index de réflexion (TS) soit déterminé avec exactitude. Nous présentons les premières mesures détaillées de la TS in situ de la morue franche (*Gadus morhua*) et du maquereau bleu (*Scomber scombrus*) dans l'Atlantique du nord-ouest. Les longueurs totales moyennes des morues et des maquereaux étudiés étaient de 52 et 38 cm, respectivement. Les distributions des TS in situ moyennes diurnes et nocturnes de morue (en mode faisceau scindé) étaient de –35,5 (b_{20} = –69,8) et –35,2 décibels (dB) (b_{20} = –69,5), respectivement. On a remarqué une stratification des TS de morue selon la profondeur la nuit puisque les TS étaient de –31,7 dB entre 140 et 220 m et de –35,8 dB entre 220 et 300 m. Nous proposons qu'un effet de profondeur sur le gonflement de la vessie natatoire expliquerait que la TS soit plus faible près du fond que dans la colonne d'eau. Les distributions des TS analysées en mode faisceau scindé et en mode faisceau simple étaient similaires, démontrant ainsi la fiabilité de notre méthode faisceau simple. La TS in situ moyenne du maquereau (mode faisceau simple) était de –56,4 dB (b_{20} = –88,0) et ne présentait pas de différence jour–nuit.

Introduction

The ratio of reflected acoustic intensity from a target to the incident intensity is generally measured in decibels (dB) and referred to as its target strength (TS). TS can be defined as

$$[1] \quad TS = 10 \log_{10} (\sigma/4\pi) = 10 \log_{10} (I_1/I_0)$$

where σ is the acoustic cross section of the target (MacLennan and Simmonds 1992, p. 165) and I_1 and I_0 are the reflected and incident intensities. The accurate determination of TS is essential to fisheries acoustics as it allows conversion of area backscattering intensity to fish biomass. When available, this parameter can be determined from the literature as a first solution; however, the most suitable TS values for application in echointegration surveys are those that reflect the specific situation of the fish under study (Foote 1987).

Foote (1991) and MacLennan and Simmonds (1992) thoroughly reviewed methods used to estimate TS that we will briefly summarize. Methods of TS estimation can be classified as either ex situ or in situ. The most widespread ex situ method involves measuring TS of caged fish of known lengths and weights (e.g., Edwards et al. 1984). By contrast, in situ methods estimate the TS of fish in their natural environment in combination with fishing. In situ methods can be further categorized as indirect or direct. With single-beam echosounders (indirect method), the beam pattern and TS effects are mixed because the position of the fish in the acoustic beam is unknown. The TS and beam pattern effects have to be separated by deconvolution (e.g., Clay 1983). Direct methods facilitate the removal of the beam pattern from single fish echoes but require dual-beam (Ehrenberg 1983) or split-beam (Foote et al. 1986) echosounders.

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The major strength of ex situ methods is that biological and behavioral characteristics (e.g., species, size, tilt angle, swimming speed, maturity, depth adaptation) of the fish under study can be precisely known (e.g., Edwards et al. 1984). On the other hand, the effects of constraining the fish on TS are unknown but possibly important, as cages may restrict swimming and hence alter tilt angle, which in turn will affect TS (Nakken and Olsen 1977). In contrast, the major advantage of in situ TS estimations is that they give estimates that account for local variations in TS owing to biological and behavioral factors and are therefore more applicable to echointegration data collected simultaneously (MacLennan and Simmonds 1992, p. 187). However, the characteristics of fish ensonified using in situ methods are often uncertain, as it is difficult to precisely match echoes and fish catch. The problem of multiple targets in the same sample volume is also a problem for in situ techniques. Another significant consideration when using in situ methods is that TS measurements have to be made on individual fish targets that may or may not be representative of most fish targets. Ensonification of schools may only yield a few individual targets at the outskirts of dense schools (e.g., Rose 1992). Indirect in situ methods are less costly, simpler to implement, and involve less potential electronic errors than direct methods, but rely on statistical methods that, by definition, carry a certain degree of uncertainty.

Atlantic cod (*Gadus morhua*) stocks are currently at record low levels in the Northwest Atlantic (Department of Fisheries and Oceans 1994). Fisheries acoustic methods, either in conjunction with or separate from existing bottom trawl surveys, may allow the development of new abundance indices independent of the fishery that may improve future cod stock assessments in eastern Canada. Acoustic abundance estimations of gadoids are being successfully conducted for cod in the Barents Sea (Godø et al. 1982; Hylen et al. 1986; Godø 1989; Godø and Wespestad 1993), and for walleye pollock (*Theragra chalcogramma*) and Pacific hake (*Merluccius productus*) in the Northeast Pacific (Kieser et al. 1987; Karp and Traynor 1989; Shaw et al. 1990; Wespestad and Megrey 1990). An important step towards developing abundance indices based on fisheries acoustics techniques for cod in the Northwest Atlantic is to determine the TS for these stocks.

There are no published reports on cod TS in the Northwest Atlantic except for a brief account that cod on the Newfoundland Shelf had a mean TS of –33.0 dB (Rose 1993). In contrast, several such studies have been published for Northeast Atlantic cod stocks. Foote (1987) reviewed in situ TS studies of gadoids and other physoclistous fishes at 38 kHz in the Northeast Atlantic and proposed that when in situ measurements are not available, TS can be approximated from body length using the following relationship:

$$[2] \quad TS = 20 \log_{10} L - 67.5$$

where L is the mean total body length (cm) of the fish (MacLennan and Simmonds 1992, p. 186), and TS is the target strength expressed in decibels (dB). The –67.5 intercept is referred to as b_{20}.

TS is also required to convert area backscatter to fish biomass in an abundance survey of Atlantic mackerel (*Scomber scombrus*) that we are currently developing in the Northwest Atlantic (Castonguay and Beaulieu 1993). No in situ TS determination has been done for mackerel prior to this study. Cage experiments with a 120-kHz echosounder yielded mean TS estimates of –57.0 dB for 31-cm mackerel ($b_{20} = -86.8$) with no day–night difference (Edwards et al. 1984). This contrasts with previous experiments by Edwards and Armstrong (1983), who found that nighttime TS of caged mackerel was lower than daytime TS by about 4 dB when expressed per kilogram of fish. This day–night difference is partly related to changes in the fish's tilt angle, as positive (head up) or negative (head down) tilt angles produce lower TS values (Nakken and Olsen 1977; Buerkle and Sreedharan 1981; Edwards et al. 1984; He and Wardle 1986; MacLennan et al. 1989). In addition, mackerel TS was recently estimated to be –56.3 dB (38-Hz echosounder and mean total fish length of 38 cm; $b_{20} = -87.9$) using the comparison method, whereby a discrete fish school of known dimensions and biomass is first ensonified before being completely fished (Misund 1994). Nakken and Olsen (1977) reported a TS estimate of –41.9 dB for caged mackerel, using a frequency of 120 kHz, but their estimate was not a mean TS but rather a mean maximum dorsal aspect TS. The dependence of TS on body length has not yet been studied in mackerel. Previous acoustic studies of mackerel distribution and abundance have used either the TS estimates of Edwards et al. (1984) (Heath 1984; Walsh and Armstrong 1985), an arbitrary TS versus body length relationship (Degnbol et al. 1988), or no TS estimate at all (Castonguay et al. 1992). Mackerel have a low TS because they do not possess a swim bladder (Collette and Nauen 1983). Foote (1980) estimated that the swim bladder is responsible for 90% of the energy reflected from fishes with swim bladders, such as cod, hence the low TS of fishes without one.

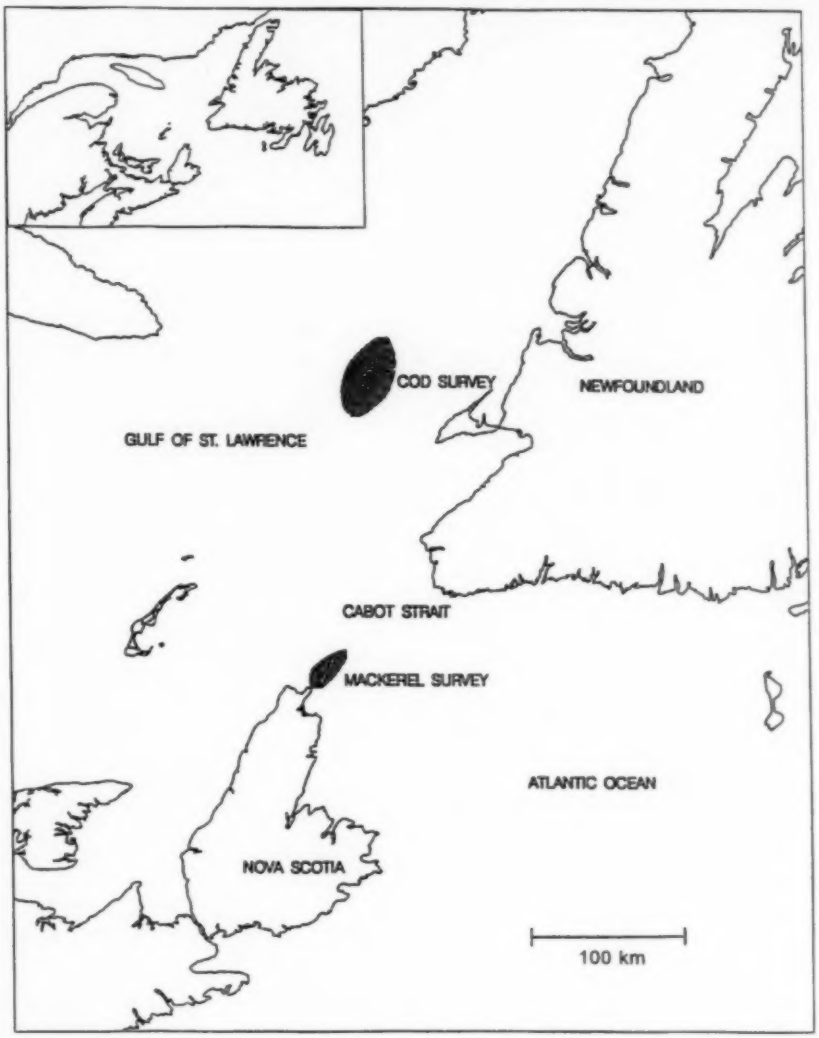
The first objective of this study was to determine daytime and nighttime in situ TS distributions of cod and mackerel that were simultaneously sampled with trawls. Through a detailed analysis of a large number of individual cod and mackerel echoes, we define appropriate daytime and nighttime TS for these species that are applicable to current (Castonguay and Beaulieu 1993) or potential (Department of Fisheries and Oceans 1991; Baird et al. 1992) hydroacoustic abundance surveys for these species in the Northwest Atlantic. Our second objective was to compare indirect and direct methods of in situ TS estimation. To accomplish this, we compared TS distributions of the same data files collected with a split-beam echosounder but that were analyzed in either single-beam or split-beam mode. To our knowledge, such a comparison between direct and indirect in situ methods, based on the same echo data files, has never been performed.

Materials and methods

Surveys

The cod survey was located in the northern Gulf of St. Lawrence from 5 to 14 May 1994 (Fig. 1). The mackerel acoustic survey was conducted in Cabot Strait from 3 to 14 June 1993 (Fig. 1). The mackerel survey has been referred to as a temporal survey (Castonguay and Beaulieu 1993) because it aims to assess the abundance of a migrating stock through a narrow migratory corridor off the southern shore of Cabot Strait. Hydroacoustic surveying was carried out in both daytime and nighttime during both surveys. The mean hydrographic conditions during each survey are shown in Table 1.

Fig. 1. Map of study area. The hatched areas indicate where each survey took place.



Data acquisition

The cod hydroacoustic sampling was carried out on board the CSS *Parizeau*, a 210-foot (1 foot = 0.305 m) oceanographic vessel equipped with a Simrad EK 500 echosounder. The echosounder used a 38-kHz, hull-mounted, 7° split-beam transducer with an output power of 2 kW. It was coupled to a hydroacoustic data processing system (HDPS, Femto Electronics Ltd.) running on a DOS-based personal computer with a global positioning system (GPS) input. Pulses 1 ms long were generated every 1.5 s. Digitized echoes (12 bits, 7500 samples/s) to which a time-varied gain (20 log R TVG) had been applied were stored on optical disks for analysis. A TS ball calibration of the echosounder using a standard copper sphere specific for the 38 kHz frequency was performed immediately prior to the survey. Ship speed during the cod survey varied from 8 to 10 knots (1 knot = 1.852 km/h).

Table 1. Mean hydrographic conditions during each survey.

	Cod survey	Mackerel survey
Surface temperature (°C)	0.3	4.9
Bottom temperature (°C)	6.0	1.0
Surface salinity (‰)	31.0	29.8
Bottom salinity (‰)	34.5	32.8
Bottom depth (m)	260	75

The CSS *F.G. Creed*, a 66-foot SWATH (small waterplane area twin-hull) vessel, was used for the mackerel survey. This vessel also used a Simrad EK 500 echosounder. Specifications, setup, and calibration were identical to those described above except for the following: 120-kHz, 1-kW split-beam

Table 2. Range of values used to select appropriate targets.

Criterion	Range of values used	Single	Split
Maximum echo duration	1.4 to 1.8 times pulse length	X	X
Depth range	11 m to bottom	X	X
Uncompensated TS	−100 to 0 dB	X	X
Phase jitter	1°		X
Maximum TS compensation	5 dB		X

transducer, 0.3-ms pulse length, and 25-kHz A/D conversion rate. Ship speed varied from 4 to 8 knots during the mackerel survey.

Both surveys used independent fishing vessels to obtain species information on observed echoes. Fishing was closely coupled with acoustic data acquisition for target interpretation. On the cod survey, fishing was conducted on board the MV *Gadus Atlantica* using an Engel-145 bottom trawl with a 5.5-m vertical opening and a 30-mm liner. Cod and redfish (*Sebastes* spp.) were the dominant species. Total catch per fish species for each tow was recorded. A subsample of the catch (≥200 fish per species) was analyzed on board (fork length, weight, sex, maturity stage, otolith extraction).

For the mackerel survey, fishing was conducted on the CSS *Calanus II* using a midwater trawl. Details of mackerel trawling are described in Beaulieu et al. (1993). Catch composition was almost exclusively mackerel and Atlantic herring (*Clupea harengus*). Fifty randomly sampled fish of each species caught were frozen for later analysis. An additional random sample of 200 fish of each species was measured (fork length), and total catch per species captured was recorded. Trawl catches and acoustic data were compared by having the acoustic vessel log echoes while steaming side by side with the deployed midwater trawl (approximately 50 m away from it). The trawl was towed about 100 m behind the trawling vessel. All these comparisons were done in daytime.

Data analysis

Data for TS analysis were collected by the HDPS from the EK 500 as individual Sv (scattering volume) values, combined with the associated angular information, at the full sample rate of the EK 500. Data were then computed back to the transducer terminals leaving only the TVG intact. No angular information was used in the selection of single-beam targets. No results were taken from TS processing done in the EK 500.

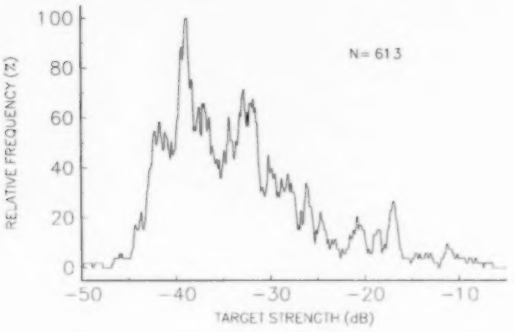
The data were first interactively edited using the HDPS to remove surface reverberation, noise, bottom, or unwanted species echoes while ensuring that all relevant data were left intact. Echoes were then converted to 40 log R for TS analysis. The second step in the analysis was to establish a target recognition template to which all echoes would be compared to verify that they were valid targets. Because a target echo is characterized in a single ping by echo duration, echo amplitude, and echo range, these parameters were used to create a footprint with which all echo patterns were compared. The echo duration (determined using the 6 dB down points) was determined by applying a window around the echo duration of a known single target (a copper sphere) using identical transceiver settings. We also compared this to the echo durations of obvious single targets at depth. The echo range normally would be limited to the depth intervals at which the study was conducted. For this study, however, the acceptance criteria were chosen between 11 to 600 m and subsequent plotting was limited to the discrete depth layers indicated. The echo amplitude was limited to those echo sequences (sequences of contiguous samples that exceeded a preselected noise threshold) with a peak amplitude yielding a TS between −100 and 0 dB. A summary for each data file indicated the number of raw echo sequences (potential targets), the number of targets that met the echo duration criteria, the portion of the remainder that met the echo range criteria, and the portion of the remainder that met the echo amplitude criteria (intermediate targets). This provided an indication of the effectiveness of the various criteria on single target selection. By far, the echo duration criterion was the most effective in removing noise and multiple targets.

Each data file was subjected to the above processing, which yielded a number of intermediate single fish targets (those common to both split-beam and single-beam analysis). Up to this point, no angle information has been used for single fish target selection. For the split-beam results only, each intermediate target was then subjected to two further criteria: phase jitter and maximum TS compensation. The phase jitter is the maximum mean sample-to-sample variation in angular position of the target. A value of 1° was used because greater values eliminated very few targets and lesser values reduced the number of targets to unacceptably low values. Single fish targets were rejected if the amount of TS compensation based on position in the beam exceeded the maximum TS compensation. Because the two-way beam angle is defined by the 6 dB down point, a value of 5 dB was chosen to ensure that the targets were well within the main lobe of the beam. The range of values used to select appropriate targets is summarized in Table 2.

No further analysis was required for split-beam targets. Single-beam targets, however, had to be further processed to remove the beam pattern effect. There are a number of single-beam processing techniques, most notably the deconvolution method of Clay (1983). For our work, we chose a simple approach to single-beam analysis. We assumed that each received echo could result from a wide range of acoustic target sizes in any position in the beam of the transducer. As an example, a returned echo equivalent to a TS of −45 dB could result from a −45 dB target on axis, or from a −43 dB target at a point where the beam attenuation is 2 dB, or from a number of other TS–position combinations. Assuming that the target has an equal probability of being in any unit pulse volume of the beam, one can statistically remove the beam pattern effect.

This method of beam pattern removal assigned discrete bins of the echo probability density function (PDF) to the target PDF weighted by the transducer beam PDF. In essence, given a received echo and the transducer PDF computed using the empirical formulation of the beam pattern by Ehrenberg (1972), one can compute a target PDF. By summing all computed target PDF values over all received echoes, one can statistically approximate the TS distribution. One restriction of this method is that the target causing a particular echo may be an extremely large target at a null in the beam. To account for this, the analysis was limited to the main lobe of the transducer. In fact, the maximum TS compensation was

Fig. 2. Distribution of in situ split-beam target strengths for an acoustic data file collected on the cod shoal in daytime (5 May 1994, 18:00 ADT) for which a comparative trawl catch was available. Frequencies are expressed as a percentage of the most frequently occurring target strength value. The mode was −38.8 dB. *N* is sample size.



limited to 5 dB to approximate the split-beam target identification criteria.

Assuming that the transducer PDF (wT) is known at each gain bin (wT_i), then for each bin of the echo PDF (wE_n) one can compute the associated target PDF (wF_n) entry using the following equation:

$$[3] \quad wF_n = \sum_{i=0}^{5dB} wE_n \cdot wT_i$$

To obtain the entire target PDF (wF) we sum over all echoes, yielding

$$[4] \quad wF = \sum_{i=1}^{n} wF_n$$

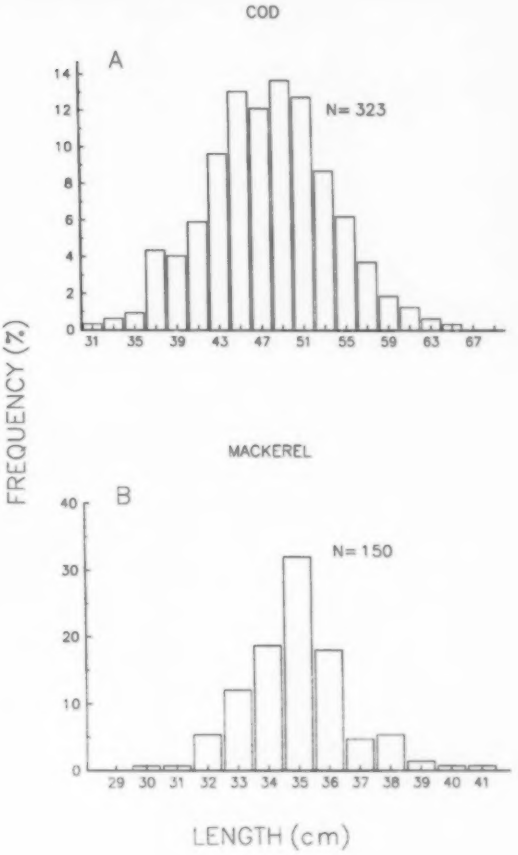
Because the split-beam method tended to reject a large number of potential targets, it was often necessary to combine a number of acoustic data files. This was not a problem with the single-beam method but for the purpose of comparing the results a number of data sets were chosen. Using this approach, our single-beam method gave a close representation of the results attained by the split-beam technique in all cases. Therefore, it was used as an approximation of the target PDF.

TS values were averaged in the linear domain for both split and single-beam data (i.e., mean acoustic cross section), as recommended by Foote (1987) and MacLennan and Simmonds (1992, p. 166). To eliminate remaining multiple targets, only targets within ±10 dB of the modal TS were selected for the calculation of the mean acoustic cross sections. We estimated that a 20-dB range would encompass most valid TS values, as observed TS values cover a large range, spanning 20 dB or more (MacLennan and Simmonds 1992, p. 184). PDF modes are indicated in the figure captions.

Both split-beam and single-beam methods for TS determination provided a TS per individual fish that was converted to TS per kilogram of fish biomass following Urick (1983, p. 415):

$$[5] \quad TS_{kg} = TS_{fish} + 10 \log_{10} (N)$$

Fig. 3. Fork length distribution of ensonified cod (A) and mackerel (B). Frequencies are expressed as a percentage of *N*, the total number of fish measured.



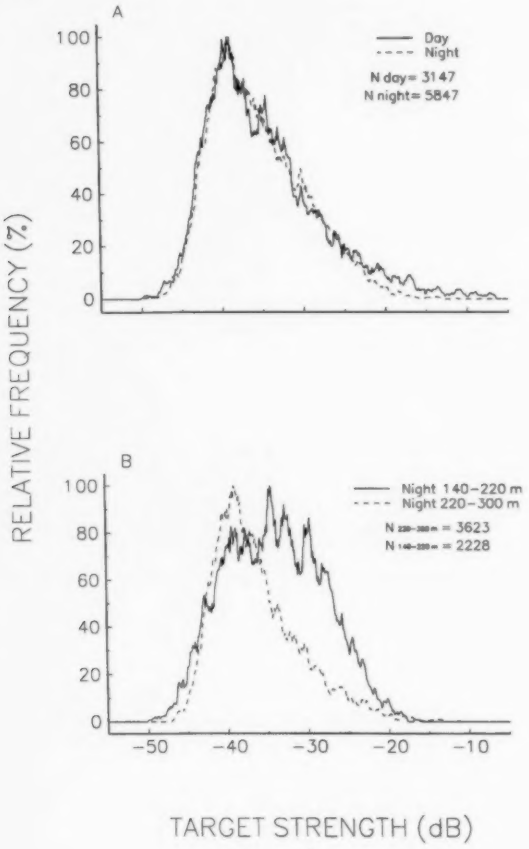
where *N* is the number of fish required for 1 kg of fish biomass (e.g., $N = 1.153$ for a mean weight of 0.867 kg).

Results

Cod

Concurrent acoustic surveying and trawling on a nearly monospecific spawning cod shoal (sensu Pitcher 1983), located at a depth of 250–300 m, provided an opportunity for characterizing cod echoes and TS with certainty. Two daytime 30-min bottom trawl sets on 5 May 1994, located at 59°48′N, 48°30′W (Fig. 1), caught 8764 kg of cod and only 397 kg of redfish. The mean in situ TS (split-beam method) of the acoustic data file logged at the same location 2 h later, also in daytime, was −34.8 dB (Fig. 2). Fork length and weight (mean ± SD) of cod randomly sampled in the two trawl sets were 47.8 ± 6.8 cm (mean total length was 51.7 cm) and 867 ± 330 g ($n = 323$) (Fig. 3A). The mean fork length of redfish in the two sets was 22.9 ± 7.1 cm (redfish were not individually weighed). The targets selected in Fig. 2 were single fish associated with the shoal.

Fig. 4. (A) Distribution of in situ split-beam target strengths for a collection of acoustic data files collected on the cod shoal by day (mode = –39.0 dB) and night (mode = –38.8 dB) (all depths considered). (B) Comparison of nighttime distributions of in situ split-beam target strengths of cod between two depth strata: 140–220 m (mode = –34.4 dB) and 220–300 m (mode = –38.8 dB). Frequencies are expressed as a percentage of the most frequently occurring target strength value. *N* is sample size.



The mean in situ TS values (split-beam method) of cod echo data files collected at or near the same site as those in Fig. 2 in the following 7 days were similar: –35.5 and –35.2 dB by day and by night, respectively (Fig. 4A). TS per kilogram of cod was –34.9 dB (b_{20} = –69.8) and –34.6 dB (b_{20} = –69.5) for day and night, respectively. These TS distributions, based on five pooled daytime and five pooled nighttime files, were determined to increase TS sample size and also to compare cod TS between day and night. Some of the single fish targets selected in daytime (Fig. 4A) were associated with the cod shoal, while others were not.

We also found evidence of a depth stratification of cod TS at night, as the mean TS between 140 and 220 m (50–130 m off-bottom) was –31.7 dB whereas it was –35.8 dB between 220 and 300 m (<50 m off-bottom) (Fig. 4B). This comparison, based on the same five nighttime data files as above, could indicate (*i*) that depth affects swim-bladder volume, and (or) (*ii*) that tilt angles are more pronounced when cod are deep, and (or) (*iii*) that large cod tend to migrate vertically at night more than small cod do. During the day, this depth-related difference in TS did not occur as cod then remained closer to the bottom. The analysis of the trawl catches lends support to the latter of the three possibilities. Indeed, cod trawled by day on the main shoal were significantly larger than cod trawled by night (47.8 cm (n = 323) versus 43.7 cm (n = 184); t test, $P < 0.001$). The percentages of immature, ripe, and spent cod in the trawl were 12.4, 82.4, and 5.2% by day and 26.1, 73.4, and 0.5% by night, respectively.

The echogram of a portion of the cod shoal in daytime is shown in Fig. 5A. This echogram represents the acoustic data file used in Fig. 2. A nighttime echogram of part of the cod shoal, logged at the same site the next day, is also shown in Fig. 5B for comparison. While most spawning cod remain near the bottom by day (although some occur as much as 75 m above the bottom), they are more dispersed at night, when some cod undergo extensive vertical migrations up to 120 m off-bottom (140 m depth).

Mackerel

The single-beam method and the split-beam method yielded similar in situ TS distributions (Fig. 6). This indicates that our single-beam method seems to effectively remove the beam-pattern effect. These comparisons between the two in situ methods were drawn from a subset of the mackerel survey comprised of 100 consecutive acoustic data files pooled together. Because mackerel target PDFs determined through the split-beam method generally had much smaller sample sizes than those determined through the single-beam method, mackerel in situ TS values will be further characterized using the single-beam method.

Results of concurrent pelagic trawling between the surface and 20 m depth and acoustic surveying were compared to identify mackerel echoes. The first trawl set, which caught 100 kg of mackerel and 300 kg of herring, had a corresponding mean in situ TS of –55.9 dB (Fig. 7A). A second series of three consecutive hauls that caught a total of 1400 kg of mackerel and 2200 kg of herring had a corresponding mean in situ TS of –55.3 dB (Fig. 7B). These TS values agree with literature values of mackerel TS. The absence of TS values in the herring range (about –42 dB) in Figs. 7A and 7B indicates that the data files contained few individual herring targets. The higher fish density of herring schools compared with those of mackerel presumably explains the absence of individual herring targets. In contrast, the third comparison between catch and TS distributions was not so straightforward: a trawl set capturing 300 kg of mackerel and 400 kg of herring yielded a comparative mean TS of –62.3 dB (Fig. 7C). In this case, targets from organisms with small TS values, which were not sampled by the trawl (presumably macrozooplankton), seem to have overwhelmed the mackerel echoes. In fact, this zooplankton target problem was especially prevalent in the top 20 m of the water column. For this reason, all TS distributions shown in Fig. 7 were from 20 to 40 m depth even though the comparative fishing was done in the top 20 m. Another reason for comparing catches in the 0–20 m range with echoes in the 20–40 m range was that the deletion of the top 11 m of echograms (owing to noise) would have left only 9 m

Fig. 5. (A) Daytime echogram of the spawning cod shoal (5 May 1994, 18:00 ADT). (B) Nighttime echogram of the same shoal (6 May 1994, 23:00 ADT). Tick marks at the top are 1 km apart whereas those on the bottom are 30 s apart. The top 100 m of the water column is not shown. The red line under the cod shoal represents the bottom.

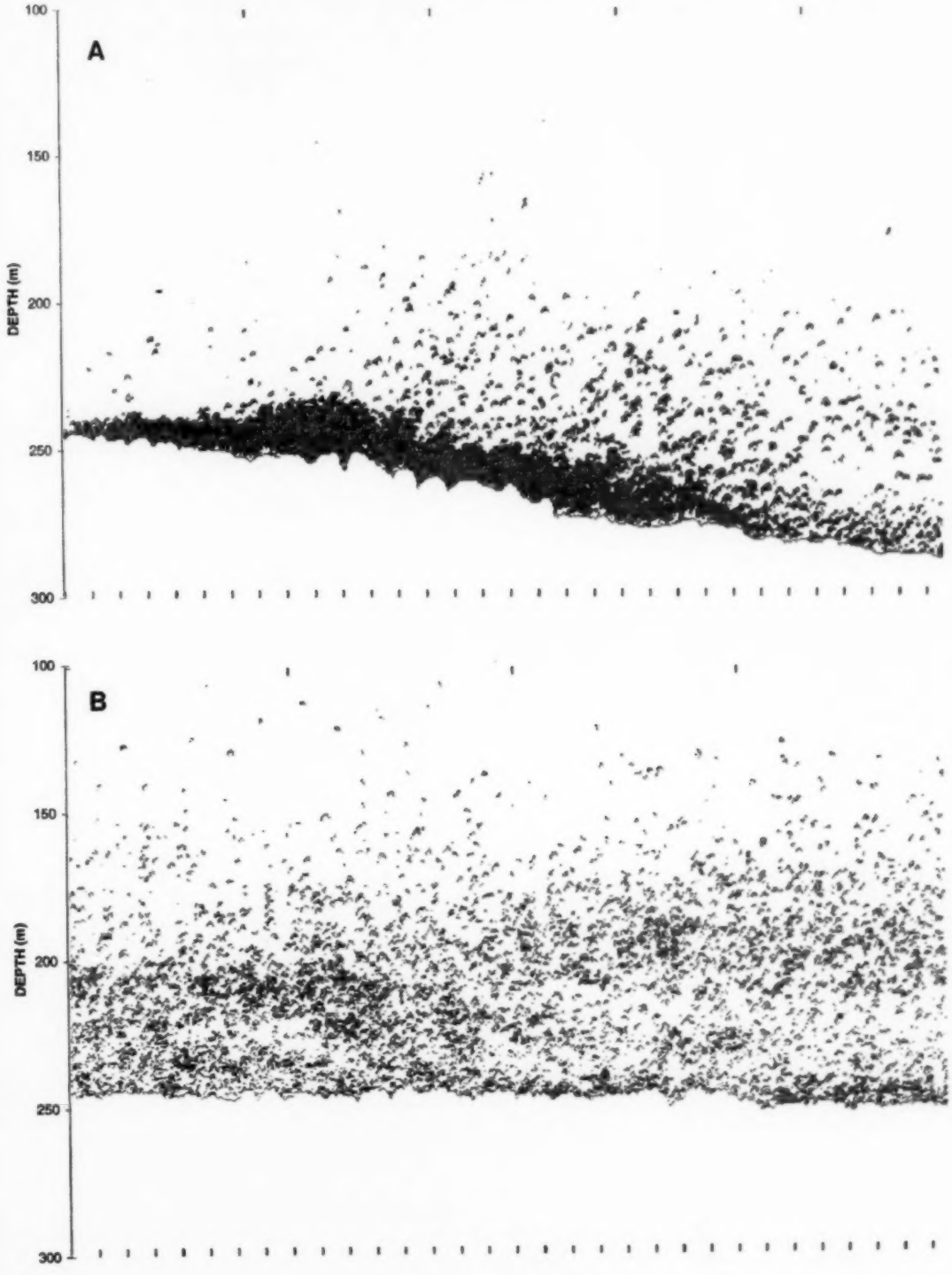


Fig. 6. Distributions of in situ target strengths collected during the mackerel survey, compared between the single-beam and the split-beam methods, and also among four depth strata labeled A, B, C, and D. Frequencies are expressed as a percentage of the most frequently occurring target strength value. *N* is sample size.

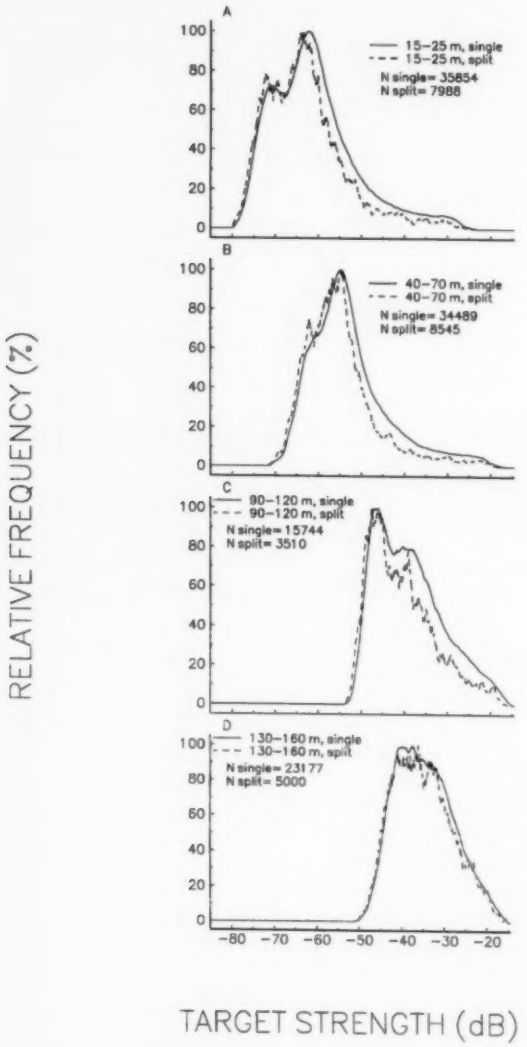
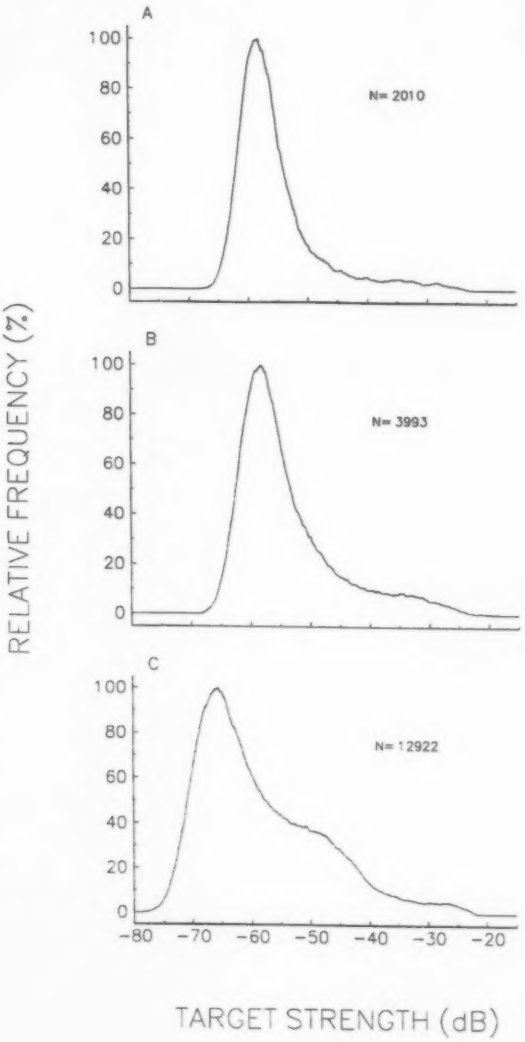


Fig. 7. Distributions of in situ single-beam target strengths for acoustic data files (labeled A, B, and C) collected during the mackerel survey for which comparative trawl catches were available. The modes (dB) were –58.1, –58.1, and –65.7 for A, B, and C, respectively. The depth range considered was 20–40 m. Frequencies are expressed as a percentage of the most frequently occurring target strength value. *N* is sample size.



(11–20 m) for the comparisons. Fork lengths of mackerel caught in hauls corresponding to TS distributions of Figs. 7A, 7B, and 7C were 35.0 ± 1.7, 34.7 ± 1.8, and 35.4 ± 2.2 cm, respectively (mean corresponding total lengths were 37.8, 37.3, and 38.2 cm, respectively). Figure 3B shows the length distribution of mackerel in the haul matching the TS distribution of Fig. 7B. Fork lengths of herring caught in hauls matching the TS distributions of Figs. 7A, 7B, and 7C were 31.2 ± 2.7, 31.5 ± 2.8, and 32.7 ± 2.7 cm, respectively.

The mean in situ TS (single-beam method) of mackerel was –56.4 dB and varied little between day and night (Fig. 8). Targets 20–40 m deep, collected on the same 6-km transect and approximately 12 h apart, were compared. The daytime mean TS was the lowest in Figs. 8A and 8B (Fig. 8A, –56.6 vs. –56.1 dB; Fig. 8B, –56.6 vs. –54.6 dB) whereas it was the highest in Fig. 8C (–56.8 vs. –57.5 dB). Hence, a TS value of –56.4 dB/individual mackerel (b_{20} = –88.0) or –52.8 dB/kg of mackerel (overall mean fork and total lengths and weight of trawled mackerel were 35.0 and 37.8 cm and 439 g) appears to be a representative value to use for

abundance assessment purposes. The 20–40 m depth stratum was selected in Fig. 8 because this is the depth stratum at which most (60%) mackerel were detected in both day and night in Cabot Strait (Castonguay and Gilbert 1995).

For the subset of 100 acoustic data files processed simultaneously (which likely include zooplankton, mackerel, herring, and maybe other targets), modal values of target PDFs increased with increasing depth, both for single-beam and split-beam data (Fig. 6). The modes were found at –62 dB between 15 and 25 m deep (Fig. 6A), at –55 dB between 40 and 70 m deep (Fig. 6B), at –46 dB between 90 and 120 m deep (Fig. 6C), and at –37 dB between 130 and 160 m deep (Fig. 6D).

Discussion

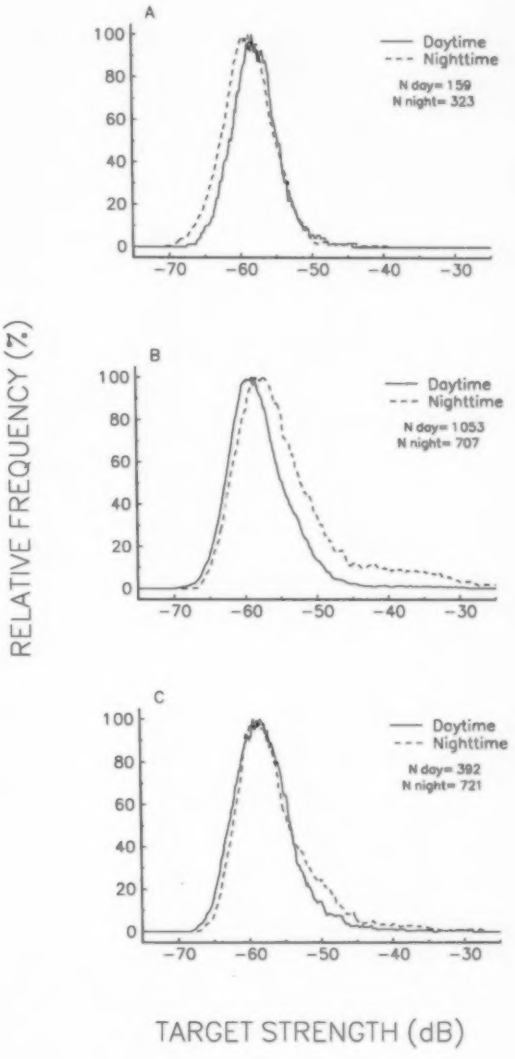
The cod TS measured in this study was 2.2 dB lower than what Foote's (1987) equation would predict. According to eq. 2, our average cod, 51.7 cm long, should have a TS of –33.2 dB whereas we found a mean TS (mean of daytime and nighttime TS values) of –35.4 dB. As Beamish (1966) previously reported, our work shows that cod remain near bottom by day and can migrate 100 m or more off-bottom at night, hence they were more densely aggregated by day than at night. This raises the question of whether those isolated targets hovering above the main shoal by day (which were selected for TS analysis) are representative of the shoal as a whole. They could be mostly from smaller fish (e.g., immature cod or redfish) that were not sampled effectively by the trawl, which would explain the low TS reported here. On the other hand, the fact that the mean TS at night, when fish are more dispersed, was almost the same as by day suggests that our daytime mean TS is valid.

The low TS of cod in this study may have been caused by their advanced stage of maturity, as ensonified cod were part of a spawning shoal. The distortion of the swim bladder caused by ripe gonads may reduce cod TS by 2–5 dB (Ona 1990). Moreover, shallow cod at night had a mean TS that was 4.1 times higher than that of deep cod. Large tilt angles would be expected when fish are swimming without a bottom reference. This may occur at night, resulting from vertical migration. If this were the case, fish would have a lower TS when higher up in the water column. However, we found the reverse. It thus seems unlikely that variations in tilt angle could explain the depth-related difference in TS.

Trawl catches indicate that cod sampled near the bottom are on average 4 cm smaller by night than by day. This argues that larger cod tend to migrate vertically at night more than small cod do (and hence become unavailable to the trawl), which may partly explain the depth-related difference in TS at night. However, according to eq. 2, a 4-cm length difference would produce only a 0.8 dB difference, hence it cannot solely explain the 4.1-dB TS difference between shallow and deep cod. It could also be that spent fish (whose swim bladders would have resumed their normal shape) tend to migrate vertically more than ripe fish do (with swim bladders distorted by gonads). The proportion of spent cod in the shoal was small (<6%), but this may also contribute slightly to the depth-related difference in TS at night because catches indicate that spent cod may migrate vertically more than ripe cod.

Cod are negatively buoyant on or near the seabed and are neutrally buoyant only at the top of their vertical range (Arnold and Greer Walker 1992). We argue that the effect of depth on swim-bladder inflation could explain most of the depth-related TS variability at night. Substantial reductions in TS produced by a reduction in swim-bladder volume may occur when cod move below the lower limit of their range of neutral buoyancy (Harden Jones and Scholes 1981). This effect, combined with

Fig. 8. Comparisons of in situ single-beam target strengths of mackerel between daytime and nighttime. Three pairs (A, B, and C) of acoustic data files are shown. Files within a pair were collected 12 h apart. Daytime and nighttime modes (dB) were as follows: A, –58.3, –59.2; B, –58.9, –58.3; C, –58.2, –58.7. The depth range considered was 20–40 m. Frequencies are expressed as a percentage of the most frequently occurring target strength value. *N* is sample size.



a swim-bladder distortion resulting from the presence of ripe gonads, could lower the TS of near-bottom cod by more than 4 dB. However, the fact that there is no TS reduction for cod found higher up in the water column (–31.7 dB versus the –33.2 dB expected value) argues that the depth-related TS difference at night is caused more by the effect of depth on swim-bladder inflation than by gonad ripeness. Unfortunately, there were not enough targets in the water column by day to make an examination of the depth dependence of TS by day possible.

The mean TS value of –56.4 dB for mackerel was determined using large sample sizes of echoes collected during an abundance index survey for this species, hence they can be applied to convert fish abundance determined from echo backscatter values into tons of fish biomass. The b_{20} value of –88.0 found in this study is within 1.2 dB of TS values previously reported for mackerel using cage experiments (Edwards et al. 1984) and the comparison method (Misund 1994). We did not detect any day–night change in the TS of wild mackerel nor did Edwards et al. (1984) or Simmonds and Armstrong (1990) for caged mackerel. In contrast, Edwards and Armstrong (1983) did report day–night differences. The absence of change in our study indicates that fish do not modify their tilt angle according to the light level in the surrounding waters. Glass et al. (1986) reported that below a certain light level, captive mackerel lose visual contact with each other; when this happens, shoaling behavior breaks down and mackerel display pronounced tilt angles. Mackerel in Cabot Strait were detected mostly between 20 and 40 m depth in day and night (Castonguay and Gilbert 1995). The absence of day–night difference in TS indicates that light levels at night at these shallow depths (half-moon present) seemed sufficient for fish to maintain visual contact with each other and hence shoaling behavior. Variable light levels at night probably explain the discrepancies among the various studies regarding diurnal variability of mackerel TS (Edwards et al. 1984; E.J. Simmonds, DAFS Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, AB9 8DB, Scotland, personal communication).

The comparison of TS PDF among depth strata (Fig. 6) suggests that the signal-to-noise ratio of weak –56 dB targets, such as mackerel, is too small to allow detection of such targets at depths greater than 90 m. It is also possible that the TS shift may be due in part to a deeper distribution of fish with higher TS, such as herring, and (or) to an increasing number of multiple targets with depth, owing to the diverging beam. The improper detection of deep mackerel may pose a problem with respect to abundance assessment because even though mackerel in Cabot Strait were detected mostly between 20 and 40 m depth (Castonguay and Gilbert 1995), there is indirect evidence that they also occur deeper in the water column. Mackerel appear to use selective tidal stream transport to enter the Gulf of St. Lawrence (Castonguay and Gilbert 1995). They would accomplish this by rising from near-bottom depths (>100 m) at slack waters created by tidal current reversals from waters flowing out of the Gulf to waters flowing into the Gulf. Hence, mackerel can probably be found at depths too great to be detected with the echosounder configuration used in 1993. Several potential ways to solve this problem need to be investigated, such as using a longer pulse length, lowering the noise margin of the echosounder, and also possibly using a 38-kHz sounding frequency in addition to or instead of the 120-kHz one that we used for this survey.

The single-beam technique used here compared favorably with the split-beam method. However, this finding does not imply that single-beam systems would provide adequate TS distributions in all cases. Further study is required to compare the performance of single- and split-beam methods. In general, split-beam systems are considerably more expensive than single-beam systems, so if the results are reasonably similar, a single-beam system may offer a more cost-effective method for most routine acoustic surveys. Furthermore, split-beam techniques can better identify multiple targets because of the increased resolution, although Fig. 2 illustrates that overlapping targets are not all eliminated by this method. Future work will attempt to improve the single-target recognition algorithm to avoid having to use a cutoff to calculate a mean TS, such as the somewhat arbitrary mode ± 10 dB criterion used in this study.

Interpreting a TS distribution by simultaneous trawling is not a straightforward task in an environment with many species present. Even though herring dominated the catch in this study, individual herring targets were not found in our TS analysis (Fig. 7). This is probably because herring schools were more densely aggregated than mackerel shoals with the result that few individual herring targets were selected. Higher net avoidance by mackerel may also partly explain why fewer mackerel were caught. In one case (Fig. 7C), most targets were from organisms having small TS values (presumably macrozooplankton), which were not sampled by our trawl. Further comparative work between trawling and acoustics is needed. For example, trawling deeper would allow comparison of the same depth strata and sampling using a plankton net may permit identification of macrozooplankton targets. These difficulties illustrate the limits of catch composition data as a tool to interpret TS distributions and echograms. As Thorne (1983) pointed out, "species information must ultimately come from capture techniques — ironically, the same techniques whose glaring deficiencies made the potential of hydroacoustics so attractive in the first place."

In conclusion, our TS measurements of spawning cod from the northern Gulf of St. Lawrence stock are a necessary first step in the development of acoustic abundance indices of cod in the Northwest Atlantic. Furthermore, we have measured a mackerel TS that is directly applicable to the acoustic abundance survey that we are currently developing for the Canadian stock of mackerel. Finally, our results indicate that simple and inexpensive single-beam echosounders may be sufficient for routine abundance surveys.

Acknowledgments

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Phosphorus–chlorophyll relationship in temperate streams and its variation with stream catchment area[1]

Erwin E. Van Nieuwenhuyse and John R. Jones

Abstract: Regression analysis of a data set compiled from the literature ($n = 292$) showed that summer mean chlorophyll concentration (Chl) among temperate streams bore a strong ($R^2 = 0.67$) curvilinear relationship with summer mean total phosphorus concentration (TP). The predicted slope of the TP–Chl relationship (Chl:TP) ranged between 0.22 at TP = 50 mg·m^{-3} and 0.08 at TP = 1030 mg·m^{-3}, the highest value of TP in our data set. A bivariate model indicated that stream catchment area had a significant effect on Chl at all levels of TP and predicted a 2.3-fold increase in Chl:TP as the stream catchment area increased from 100 to 100 000 km^2. We suggest that TP may provide a reliable basis for predicting Chl in small and large temperate streams worldwide.

Résumé : Le calcul de régression d'une série de données tirées de la littérature ($n = 292$) a montré que la concentration estivale moyenne de chlorophylle (Chl) dans les cours d'eau tempérés présentait une forte ($R^2 = 0{,}67$) relation curviligne avec la concentration estivale moyenne du phosphore total (PT). La pente prévue de la relation PT–Chl (Chl:PT) allait de 0,22 pour PT = 50 mg·m^{-3} à 0,08 pour PT = 1030 mg·m^{-3}, valeur la plus élevée de PT dans notre série de données. Un modèle à deux variables a indiqué que la superficie du bassin versant du cours d'eau avait un effet significatif sur Chl à toutes les concentrations de PT, et a prédit une augmentation d'un ordre de 2,3 du rapport Chl:PT si la superficie du bassin versant du cours augmentait de 100 à 100 000 km^2. Nous pensons que le phosphore total peut constituer une base fiable pour prédire la concentration de chlorophylle dans les cours d'eau petits et grands des régions tempérées du monde entier.
[Traduit par la Rédaction]

Introduction

A generally positive relationship between seasonal or annual mean chlorophyll (Chl) and total phosphorus concentration (TP) is well documented in temperate and subtropical lakes and reservoirs (Jones and Bachmann 1976; Canfield 1983; Hoyer and Jones 1983; Prepas and Trew 1983). Recent lake studies confirm that the TP–Chl relationship is curvilinear over broad ranges of TP values (Straskraba 1980, 1985; Prairie et al. 1989; Jones and Knowlton 1993) and that the slope or intercept of this relationship may vary systematically with some coregulatory factor, especially mean total nitrogen concentration (Smith 1982; Canfield 1983; McCauley et al. 1989) or the ratio of euphotic depth to mean depth (Verduin et al. 1978; Straskraba 1980). Such studies have increased understanding of the relationship between P supply and algal productivity in lentic ecosystems (Schindler 1978; Golterman and Kouwe 1980) and led to the development of predictive models for managing lake and reservoir water quality (Vollenweider and Kerekes 1980; Peters 1986; Walker 1986). Much less is known about the phosphorus–chlorophyll relationship in streams.

Previous studies indicate that Chl or other measures of mean sestonic algal abundance may indeed bear a positive relationship with TP or other measures of average P concentration in some streams (Nusch 1978; Jones et al. 1984; Shehata and Bader 1985; Soballe and Kimmel 1987; LaPerriere et al. 1989). In P-rich streams, however, average abundance of sestonic algae may not be correlated with average P or N concentration (Kilkus et al. 1975; Baker and Kromer-Baker 1979; Straskraba 1985; Bennet et al. 1986). Among these systems, mean sestonic algal abundance may vary instead with stream catchment area, mean depth, mean hydraulic flushing rate, or other physical factors (Kilkus et al. 1975; Baker and Kromer-Baker 1979; Bennet et al. 1986; Soballe and Kimmel 1987). Collectively, these studies suggest that the TP–Chl relationship in streams may, as in lakes, be curvilinear and that it likely varies systematically with some physical factor. To test this hypothesis, we analyzed the TP–Chl relationship for temperate streams on four continents (most in North America) and determined how this relationship varied with stream catchment area.

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Table 1. Summary statistics for summer (May–September) mean total phosphorus and chlorophyll concentrations and stream catchment area among temperate streams (n = 292).

Variable	Mean ± SE	Min.–Max.	Percentile		
			25%	50%	75%
Total P (mg P·m^{-3})	192±12	5–1030	48	100	263
Chlorophyll (mg·m^{-3})	27±1.8	0.4–170	4.9	17	35
Catchment area (km^2)	33 060 ± 3962	1 – 541 310	450	8130	42 000

Methods

Concurrent estimates of TP and Chl (n = 292) in 115 north temperate streams and 1 south temperate stream were compiled from 24 published or unpublished sources.[3] Most of the data set described North American streams (n = 231), especially tributaries of the Missouri or Lower Mississippi rivers (n = 116, Smart 1980; Smart et al. 1985; Soballe and Threlkeld *1985; Lohman 1988; Skadland 1992; Van Nieuwenhuyse* 1993), the Upper Mississippi River (n = 65, Soballe and Bachmann 1984; Metropolitan Council, Environmental Division 1976–1992; Greg Payne, Water Resources Division, U.S. Geological Survey, Mounds View, Minn., unpublished data), and Hudson Bay (n = 49, unpublished data from routine water quality monitoring program, available from Gary Dunn, Prairie Provinces Water Board, Room 201, 2050 Cornwall Street, Regina, SK S4P 2K5). Data for European streams (n = 48) included the Rhine River at Lobith, the Meuse River at Eijsden (unpublished data from routine water quality monitoring program, 1977–1993, available from RIZA (Dutch Governmental Institute on Inland Water Management and Waste Water Treatment), P.O. Box 10, 8200 AA Lelystad, The Netherlands), and the Ruhr River near Essen (Nusch 1978; Albrecht 1988). Also represented were the Nakdong River in Korea (n = 2, Choi and Park 1986), the Shatt Al-Arab River in Iraq (n = 1, Al-Saadi and Antoine 1981), and the Cox River in New South Wales, Australia (n = 10, Ferris and Tyler 1985). In all, 15 sites had ≥5 years of observations. Small streams sampled within a few kilometres of treated sewage outfalls (e.g., Wisniewski et al. 1985) were not included in our data set.

Each value of TP and Chl was calculated as the arithmetic mean of all samples collected at a single location during summer months (May–September), regardless of flow conditions. The sampling regime at all locations was periodic, with frequencies ranging from monthly (n = 49) to biweekly (n = 191) or weekly (n = 52). Total P concentration was measured using standard methods of chemical digestion and phosphate-P determination (e.g., Prepas and Rigler 1982). Chlorophyll concentrations were uncorrected for phaeophytin. Values of catchment area were taken directly from published sources (n = 274) or estimated by planimetry from scaled figures or maps.

All statistical analyses were conducted using $\log_{10}$-transformed values to help stabilize variance. Robust locally weighted sequential smoothing (LOWESS, Cleveland 1979) was used to provide an initial, model-free assessment of the form of the relationship between two variables (Prairie et al. 1989). Parameters used in all LOWESS analyses were $\delta = 0$, n – steps = 2, and $f = 0.5$. All interaction terms evaluated in multiple regression analyses were assumed to be multiplicative (McCauley et al. 1989). Antilogs of log Chl values calculated from regression models were corrected for transformation bias by multiplying the antilogs by e^c, where $c = 2.65 \cdot s^2$ and s = standard error of regression in $\log_{10}$ units (Ferguson 1986). Correlation coefficients were compared using Fisher's Z transformation and Student's t test (Zar 1974).

[3] Data may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada.

Results

Phosphorus concentration

The average total phosphorus concentration (TP) ranged between 5 and 1030 mg·m^{-3}, with a grand mean value of 192 mg·m^{-3} (Table 1). Most (65%) TP values, however, ranged between 50 and 500 mg·m^{-3}. Systems with TP < 50 mg·m^{-3} (n = 79) included some two dozen streams draining predominantly (>65% of land cover) forested catchments in south-central Missouri (e.g., Smart et al. 1985), the Cox River in Australia, and the Beaver, Battle, Carrot, Red Deer, and North and South Saskatchewan rivers in west-central Canada. Extremely fertile streams (TP > 500 mg·m^{-3}, n = 38) drained either densely populated catchments (n = 28), such as the Ruhr, Meuse, and Lower Rhine in northern Europe, or rural catchments where modern agriculture accounted for >80% of land cover (n = 10), such as the Minnesota, Grand, and Chariton rivers in the midwestern United States. Interannual variation in TP, as estimated by the coefficient of variation in mean TP among years at a site (CV_{TP}), ranged between 19 and 67% among sites for which ≥5 years of data were available. The median value of CV_{TP} was 35%.

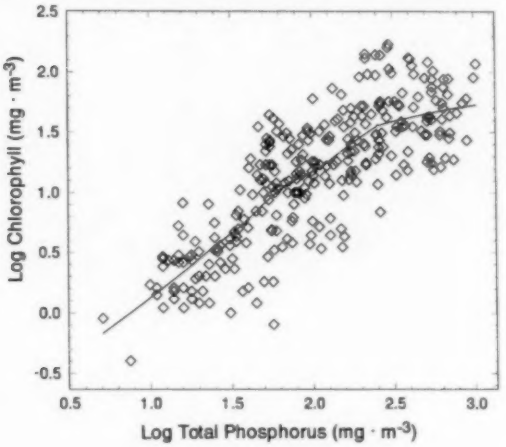
Chlorophyll concentration

The average Chl ranged between 0.4 and 170 mg·m^{-3}, with a grand mean value of 27 mg·m^{-3} (Table 1). The lowest Chl value was in Clifty Creek, a 20-km^2 mostly (>90%) forested catchment in south-central Missouri (Lohman 1988). The highest value occurred in the Minnesota River at Jordan, a 42 000-km^2 agricultural catchment in southern Minnesota (Metropolitan Council, Environmental Services Division 1987; Payne 1994). Most (65%) Chl values, however, ranged between 5 and 65 mg·m^{-3}. At sites where ≥5 years of data were available, interannual variation in mean Chl (CV_{Chl}) ranged between 21 and 116%, with a median value of 45%.

Phosphorus–chlorophyll relationship

Analysis of the data using LOWESS suggested that the relationship between log TP and log Chl was slightly curvilinear, but not sigmoidal (Fig. 1). This observation was supported by

Fig. 1. Relationship between log total phosphorus and log chlorophyll concentrations among temperate streams. Each observation (n = 292) is the arithmetic mean of ≥5 samples collected periodically over a given summer (May–September). The trend line, estimated by robust locally weighted sequential smoothing (LOWESS), suggests a curvilinear relationship.



regression analysis. A quadratic model explained 3% more variance in log Chl than a simple linear model, whereas adding a third-order term had no effect on residual error (t = –0.34, p = 0.73). The TP–Chl relation in temperate streams could thus be approximated as

[1] $$\text{Log Chl} = -1.65 + 1.99(\text{Log TP}) - 0.28(\text{Log TP})^2$$
$$(s = 0.32, R^2 = 0.67, F = 291, p < 0.001, n = 292)$$

This model (Fig. 2) indicated that the slope of the TP–Chl relationship (Chl:TP) decreased with increasing TP, attaining a maximum value of 0.22 at TP = 50 mg·m^{-3} and a minimum value of 0.08 at TP = 1030 mg·m^{-3}, the highest value of TP in our data set.

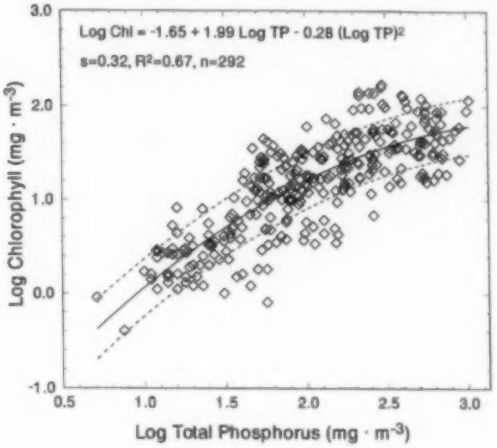
Effect of stream catchment area

Stream catchment area (A_c) ranged between 1 and 541 000 km^2 with a grand mean value of 33 060 km^2 (Table 1). The four largest catchments were the Shatt Al-Arab near Basrah (541 300 km^2), the Saskatchewan near The Pas, Manitoba (328 000 km^2), the Vistula at Warsaw (194 000 km^2), and the Rhine at Lobith (160 000 km^2). Sixty-five percent of observations, however, described streams with catchment areas ranging from 450 to 66 000 km^2.

There was a weak, but significant (r = 0.44, $p < 0.0001$) positive correlation between log A_c and log TP. An even stronger correlation (r = 0.54) was found between log A_c and log Chl (Fig. 3). Residual values calculated from eq. 1, when plotted as a function of log A_c (Fig. 4), indicated that eq. 1 overestimated log Chl in streams with small catchments while underestimating log Chl in large systems. These results suggested that stream catchment size might affect at least one of the three coefficients in the quadratic model.

This hypothesis was supported by multiple regression analysis. A series of regression models including log A_c and terms representing potential interaction effects with log TP or

Fig. 2. Log–log relation between mean total phosphorus concentration and mean chlorophyll concentration among streams (eq. 1). Dashed curves approximate 65% confidence interval for individual predicted values.



(log TP)^2 indicated that log A_c exerted a positive effect on the intercept of eq. 1 (t = 8.14, $p < 0.0001$), but had no effect on the coefficient of the linear or quadratic term ($t < 1.19$, $p > 0.11$). Consequently, the effect of stream catchment area on the TP–Chl relationship in streams could be approximated as

[2] $$\text{Log Chl} = -1.92 + 1.96(\text{Log TP}) - 0.30(\text{Log TP})^2 + 0.12(\text{Log } A_c)$$
$$(s = 0.29, R^2 = 0.73, F = 261, p < 0.0001, n = 292)$$

This model accounted for 18% of the variance in log Chl not explained by eq. 1 and indicated that the slope of the TP–Chl relationship at all levels of TP increased some 2.3-fold as A_c increased from 100 to 100 000 km^2 (Fig. 5).

Discussion

Our study confirms that there is a generally positive relationship between Chl and TP in temperate streams and that this relationship is curvilinear (Fig. 2). Moreover, the average precision (standard error of regression) of our stream model (0.32) falls well within the range of standard errors (0.23–0.53) associated with large-scale lake models (Jones and Bachmann 1976; Schindler 1978; Soballe and Kimmel 1987). Similarly, among-year variation in TP (19–67%) and Chl (21–116%) for systems with records for ≥5 years compares favorably with estimates of CV_{TP} (2–80%) and CV_{Chl} (0–400%) documented for lakes and reservoirs (e.g., Knowlton et al. 1984). These results support the view that lotic and lentic ecosystems respond similarly to P enrichment (Jones et al. 1984; Straskraba 1985) and that TP may provide a reliable basis for predicting the average abundance of sestonic algae in many temperate streams worldwide.

Specifically how increasing P concentration leads to increased Chl in streams remains unclear. Assuming, however, that Chl provides an index of mean annual productivity in aquatic ecosystems (Swanson and Bachmann 1976; Schindler

Fig. 3. Scatter plot and LOWESS trend line illustrating positive correlation between log stream catchment area and (*a*) log total phosphorus concentration or (*b*) log chlorophyll concentration. The correlation between area and chlorophyll concentration is stronger than between area and total phosphorus concentration (Fisher's $Z = 2.31$, $p = 0.02$).

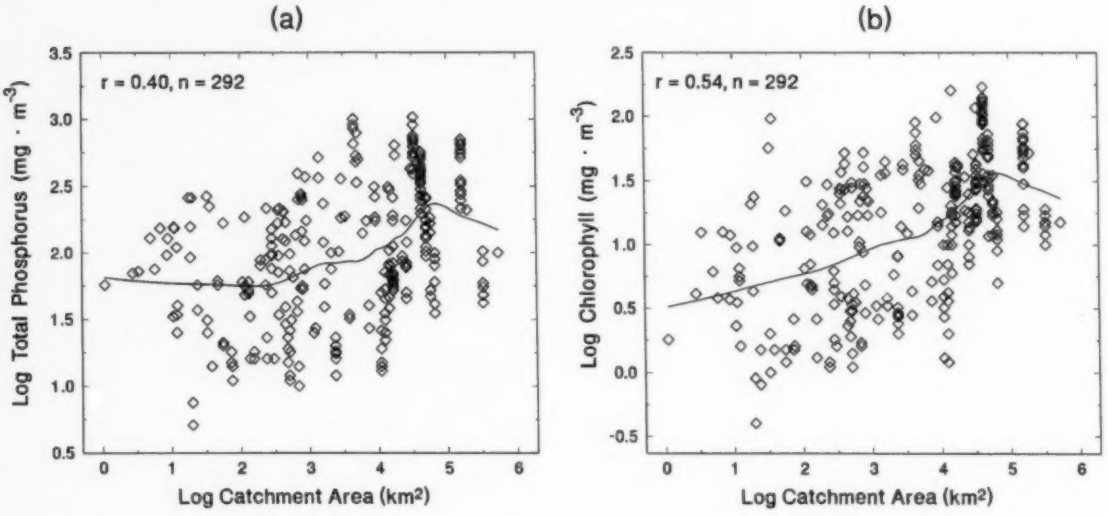


Fig. 4. Residual values of phosphorus–chlorophyll regression model for temperate streams (Fig. 2) plotted against log stream catchment area (A_c). The LOWESS trend line suggests that the univariate model (eq. 1) overestimates log Chl in catchments <7000 km^2 while underestimating log Chl in larger catchments.

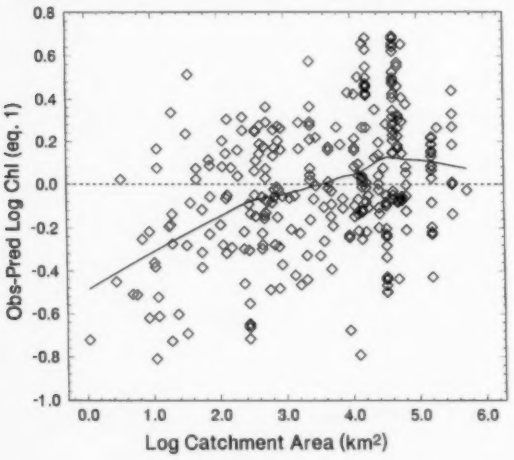
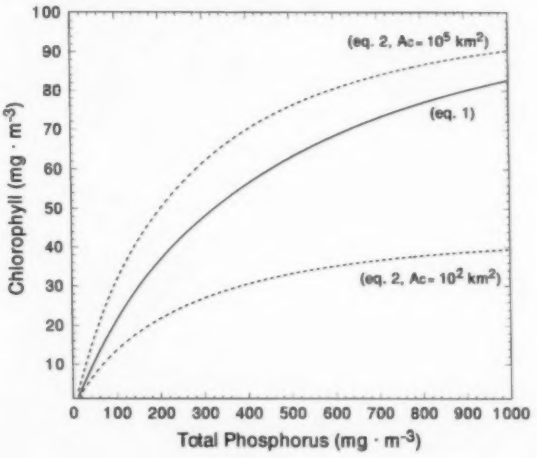


Fig. 5. Arithmetic-scale plot of the phosphorus–chlorophyll relationship in streams and its variation with stream catchment area (A_c). The solid curve shows the predicted relationship for streams regardless of A_c (eq. 1); the broken curves show the predicted relationship for streams of widely differing A_c (100 000 and 100 km^2). Values are corrected for transformation bias (see Methods).



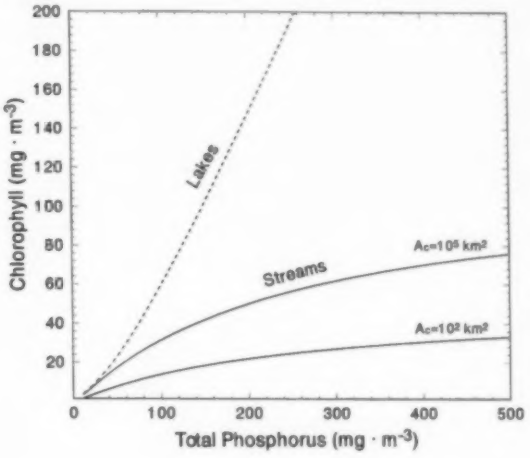
1978; Smith 1979; Golterman and Kouwe 1980), at least two general mechanisms may be postulated. First, P enrichment may stimulate algal growth directly in the water column, where Chl is measured. This mechanism presumably accounts for water blooms or other consequences of enhanced plankton productivity normally associated with P-rich lakes (Jones and Bachmann 1975; Kalff 1983; Ramberg 1988), but also well documented in streams, especially during dry-weather seasons when discharge is relatively low (Talling and Rzoska 1967; Kilkus et al. 1975; Lack et al. 1978; Nusch 1978; Meybeck et al. 1988; Cullen 1993). Alternatively, P enrichment may first stimulate algal growth on the streambed (Aizaki and Sakamoto 1988; Biggs and Close 1989; Lohman et al. 1992), leading to nuisance levels of benthic biomass under low flow conditions (Welch et al. 1988). This enhanced benthic productivity would likely also lead to increased abundance of sestonic algae because benthic algae are often sloughed into suspension as pseudoplankton under low flow conditions (Butcher 1932;

Blum 1956; Swanson and Bachmann 1976). Moreover, any benthic biomass not lost to sloughing, decomposition, or grazing under these comparatively benign hydraulic circumstances would be subject to severe scour and washout during subsequent high flow events (Dauta 1975; Swanson and Bachmann 1976; Tett et al. 1978; Jones et al. 1984; LaPerriere et al. 1989; Lohman et al. 1992). Thus, P enrichment may result in increased Chl regardless of whether the predominant mode of algal production in a particular stream is planktonic or benthic or some mixture of the two.

The positive effect of stream catchment area on Chl:TP (Fig. 4) suggests that physical factors may coregulate Chl at all levels of TP. Among the many factors known to vary predictably with stream catchment area (Dunne and Leopold 1978), mean hydraulic flushing rate (ρ) is perhaps the most important in determining sestonic algal abundance (Kilkus et al. 1975; Soballe and Kimmel 1987). The importance of ρ in coregulating Chl stems from its effect on the sestonic algal washout rate (Brook and Woodward 1956; Mann 1972; Sivko et al. 1972; Lack et al. 1978; Imhoff and Albrecht 1982; Soballe and Bachmann 1984; Soballe and Threlkeld 1985). In streams draining small catchments (≈100 km^2), ρ may average 1.0 d^{-1} or more, depending on the hydrologic region; whereas in a typical large system (≈100 000 km^2), ρ might drop to 0.1 d^{-1} or less (Talling and Rzoska 1967; Capblancq and Dauta 1978; Keup 1985; Ruyter van Steveninck et al. 1992; Van Nieuwenhuyse 1993). This 10-fold reduction in average flushing rate would substantially reduce the average washout rate imposed on sestonic biomass, given that the immersed weight of sestonic biomass (like all seston) is supported entirely by the water column (Eddy 1934; Ketchum 1954; Margalef 1960; Lack et al. 1978; Jones 1984). This reduced washout rate may help increase Chl directly, by allowing more time for algal photosynthesis, nutrient uptake, and cell reproduction to proceed in suspension (Talling and Rzoska 1967), or indirectly, by providing more time for dislodged benthic algae (pseudoplankton) to accumulate passively in the water column (Swanson and Bachmann 1976). Either way, Chl:TP may increase with stream catchment area in part because the average loss (washout) rate a stream imposes on sestonic algal biomass generally decreases with catchment size.

Our results also confirm previous studies (Burkholder-Crecco and Bachmann 1979; Jones et al. 1984; Soballe and Kimmel 1987) indicating that the average abundance of sestonic algae per unit TP is generally lower in streams than in lakes. According to our bivariate model for streams (eq. 2), long-term average Chl:TP near the mouth of a moderately fertile (TP = 100 mg·m^{-3}), 100 000-km^2 catchment would be 0.31. This value is about half of what would be predicted for a P-limited lake of comparable TP (McCauley et al. 1989). This disparity in Chl:TP between large streams and P-limited lakes is about the same as between small streams and large streams (Fig. 6). If the latter difference is indeed influenced by the differential loss (washout) rate of sestonic algae, then so may the former. According to this logic, Chl:TP may be lower in streams than in lakes in part because streams impose a more rapid average loss rate on sestonic algal biomass than lakes do. Low Chl:TP in lakes, however, is often associated with low nitrogen to phosphorus ratios (McCauley et al. 1989), low ratios of euphotic depth to mean depth (Verduin et al. 1978), or high concentrations of suspended solids (Jones and Knowlton 1993). Variation in Chl:TP among inland waters may thus owe more to factors influencing mean algal growth rate (Smith 1982) than to factors regulating mean algal loss rate.

Fig. 6. Comparison of the phosphorus–chlorophyll relationship in temperate streams and lakes. Solid curves show predicted Chl concentrations (eq. 2) in streams of widely differing catchment area (100 000 and 100 km^2). The broken curve depicts the trajectory of expected Chl concentration in P-limited lakes (i.e., total nitrogen to TP ratio = 25, Forsberg and Ryding 1980) and is calculated from McCauley et al.'s (1989) model on the basis of data compiled from the literature (n = 382, R^2 = 0.80, s = 0.23 assumed for bias correction).



In conclusion, our study indicates that there is a generally positive relationship between Chl and TP in temperate streams and that Chl:TP generally increases with stream catchment area. These findings suggest that TP may provide a reliable basis for predicting average abundance of sestonic algae in temperate streams great and small.

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Relationships between geographic distribution and abundance of American plaice (*Hippoglossoides platessoides*) in the southern Gulf of St. Lawrence

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Abstract: Optimal foraging theory predicts the expansion of population range into marginal habitat as abundance increases; percent changes in local density should be greater in marginal than in optimal habitat as population size changes. Using data from annual bottom trawl surveys (1971–1992), we found that the abundance of American plaice (*Hippoglossoides platessoides*) in the southern Gulf of St. Lawrence varied fivefold during this period, but its geographic range (area containing 95% of the population) showed no tendency to expand as population size increased. The area of highest density (area containing 50% of the population) did show a slight tendency to expand with population size, but contrary to predictions, there was no tendency for changes in density to be proportionally greater in marginal areas where plaice were uncommon. Slight but significant ($P < 0.05$) differences in geographic distribution occurred between periods of low and high abundance but accounted for only a small proportion of the total variation in catch rates. Average catch rates were highest in the same areas in both abundance periods, and no large shifts in distribution occurred between the two periods. These results contrast with published results for Atlantic cod in the southern Gulf of St. Lawrence.

Résumé : Selon la théorie de la recherche optimale de nourriture, l'aire d'une population s'étendrait dans son habitat marginal à mesure que s'accroît l'abondance; les changements en pourcentage de la densité locale devraient être plus forts dans l'habitat marginal que dans l'habitat optimal quand la taille de la population change. À partir de données recueillies pendant des campagnes annuelles de chalutage sur le fond (1971–1992), nous avons trouvé que l'abondance de plie canadienne (*Hippoglossoides platessoides*) dans le sud du golfe du Saint-Laurent a varié d'un facteur de 5 pendant cette période, mais que son aire géographique (zone contenant 95% de la population) n'a montré aucune tendance à l'expansion quand la taille de la population augmentait. La zone de densité la plus forte (contenant 50% de la population) a bien montré une légère tendance à s'étendre en fonction de la taille de la population mais, contrairement aux prévisions, aucune tendance ne montrait que les changements de la densité seraient proportionnellement plus grands dans les zones marginales où les plies étaient peu communes. Des différences légères mais significatives ($P < 0{,}05$) dans la distribution géographique se sont produites entre les périodes de faible et de forte abondance, mais elles n'expliquaient qu'une petite proportion de la variation totale des taux de capture. Les taux moyens de capture étaient les plus hauts dans les mêmes régions aux deux périodes d'abondance, et aucun grand changement de la distribution ne s'est produit entre ces deux périodes. Ces résultats contredisent les données publiées sur la morue franche dans le sud du golfe du Saint-Laurent.
[Traduit par la Rédaction]

Introduction

Optimal foraging theory has played a central role in the interpretation of ecological data in recent decades (e.g., Stephens and Krebs 1986). This theory assumes that animals adopt the foraging strategy that maximizes their net energy intake per unit time (or, more generally, that maximizes their fitness). One application of optimal foraging theory has been the theory of density-dependent habitat selection (Fretwell and Lucas 1970; reviewed by Rosenzweig 1991). This theory predicts the distribution of foragers among habitats assuming an ideal free distribution (IFD) (i.e., assuming that foragers have ideal knowledge of their environment and are free to move among habitats). If foragers have equal competitive abilities, then the IFD predicts that they should distribute themselves among habitats so that profit rate (e.g., net energy uptake rate, fitness) is equal among all occupied habitats. At low abundance, only the best habitats should be occupied. As abundance increases, foragers should begin to occupy poorer habitats as profit rates in the better habitats decrease because of competition. Thus,

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the distribution of foragers among habitats is predicted to be density dependent.

MacCall (1990) recently applied the theory of density-dependent habitat selection to the large-scale geographic distribution of marine fish populations. A prediction was that population range should expand as population size increases. This range expansion is expected when individuals begin to occupy poorer habitats as abundance and competition increase in the better habitats.

This prediction has important implications for fisheries management and stock assessment. Catchability, the proportion of a population captured by a unit of effort, is expected to be inversely proportional to stock area in the case of commercial fisheries (Paloheimo and Dickie 1964). Thus, if stock area is proportional to population size, catchability will increase as abundance decreases. An inverse relationship between catchability and population size has been widely observed in marine fisheries (e.g., Winters and Wheeler 1985, and references therein; Crecco and Overholtz 1990, and references therein; Rose and Leggett 1991). Winters and Wheeler (1985) concluded that an inverse relationship between catchability and stock abundance "may be a general feature of marine fish populations," probably reflecting "systematic changes in stock area." Crecco and Overholtz (1990) concurred that it is unlikely that distributional area remains uniform as stock abundance changes. Direct tests have usually supported the prediction of a positive correlation between stock area and abundance (e.g., Winters and Wheeler 1985; Crecco and Overholtz 1990; MacCall 1990; Rose and Leggett 1991; Swain and Wade 1993; Marshall and Frank 1994; Swain and Sinclair 1994), though there have been exceptions to this rule (Murawski and Finn 1988; Marshall and Frank 1994). Indices of stock area used in these tests have suffered from a number of defects, in particular a tendency to increase with abundance even when changes in abundance are spatially uniform (Marshall and Frank 1994; Swain and Sinclair 1994). Swain and Sinclair (1994) described an index of stock area that depends only on the spatial spread of fish distribution. Using this statistic, they confirmed that population range increased with abundance for Atlantic cod, *Gadus morhua*, in the southern Gulf of St. Lawrence.

A second approach to testing for density-dependent geographic responses by marine fishes has been to test for regional variation in rates of local density change with changes in population size (Myers and Stokes 1989; Swain and Wade 1993; Marshall and Frank 1995). If habitat selection is density dependent and geographic range expands as population size increases, then as population size changes the percent changes in local density should be greater in marginal than in optimal habitat (Marshall and Frank 1995, see their Fig. 3). This prediction has been supported in most cases examined. As cod abundance increased in the southern Gulf of St. Lawrence, density increased slowly in the regions where cod were concentrated at low population sizes and more rapidly in surrounding regions (Swain and Wade 1993). Similarly, as the population size of juvenile haddock, *Melanogrammus aeglefinus*, varied on the Scotian Shelf, local abundance changed more rapidly in areas where haddock were scarce than in areas where they were abundant (Marshall and Frank 1995). However, such regional variation in rates of local density change can reflect density-dependent shifts in the center of distribution as well as range expansion or contraction. For example, for cod older than age 3, Swain and Wade (1993) found that the areas of highest density shifted from the regions preferred at low abundance to surrounding regions at high abundance. This shift in geographic distribution may reflect density-dependent changes in temperature selection by cod (Swain and Kramer 1995).

In this paper, we test effects of population abundance on the geographic distribution of American plaice, *Hippoglossoides platessoides*, in the southern Gulf of St. Lawrence. We examine three aspects of distribution. First, we test for effects of population size on geographic range. Second, we test for regional variation in rates of local density change with changes in population abundance. Third, we test for shifts in geographic distribution between periods of low and high abundance. Finally, we discuss the results of these tests with respect to optimal foraging theory and the determinants of habitat quality for American plaice.

Material and methods

Study area and population

The southern Gulf of St. Lawrence consists of a shallow shelf, the Magdalen Shallows, bordered on the north by a 500-m trench, the Laurentian Channel (Fig. 1). The physical oceanography of the area is described by Strain (1988) and Koutitonsky and Bugden (1991). In summer and early autumn, bottom temperatures are typically below 2°C over most of the Magdalen Shallows, increasing shoreward and with depth along the Laurentian Channel (e.g., Chouinard and Swain 1994).

The biology of American plaice in the southern Gulf of St. Lawrence is described by Powles (1965). Male plaice have a slower growth rate and mature at an earlier age and smaller size than do female plaice. Plaice migrate between feeding grounds occupied on the Magdalen Shallows (40 to 100 m, −1.0 to 0.0°C) in summer and autumn and offshore overwintering grounds along the Laurentian Channel (185 to 460 m, 2.0 to 5.0°C; Powles 1965). Powles (1965) recognized two groups of plaice in the southern Gulf in summer: a northwest group occupying Chaleur Bay and the western Shallows from the Shediac Valley to the Orphan Bank area, and a southeast group occupying areas off the west coast of Cape Breton Island. Tagging studies revealed little movement of adults between the two areas, but meristic studies failed to reveal differences between the two areas, perhaps reflecting mixing via larval drift. In a recent study of allozyme variation and of restriction fragment length polymorphisms in mitochondrial DNA, Stott et al. (1992) found no evidence of population subdivision in the Gulf of St. Lawrence. Since 1971, the overall abundance of plaice in the southern Gulf has varied fivefold and individual age-classes have ranged in abundance between 5- and 15-fold. Mean catch rates in annual bottom trawl surveys of the southern Gulf were as high as 900–1000 plaice/tow in the mid to late 1970s and as low as 200–250 plaice/tow in the mid to late 1980s (Fig. 2).

Data sources

A bottom trawl survey of the southern Gulf of St. Lawrence

Fig. 1. Map of the southern Gulf of St. Lawrence, showing bathymetry and strata used in the September bottom trawl surveys.

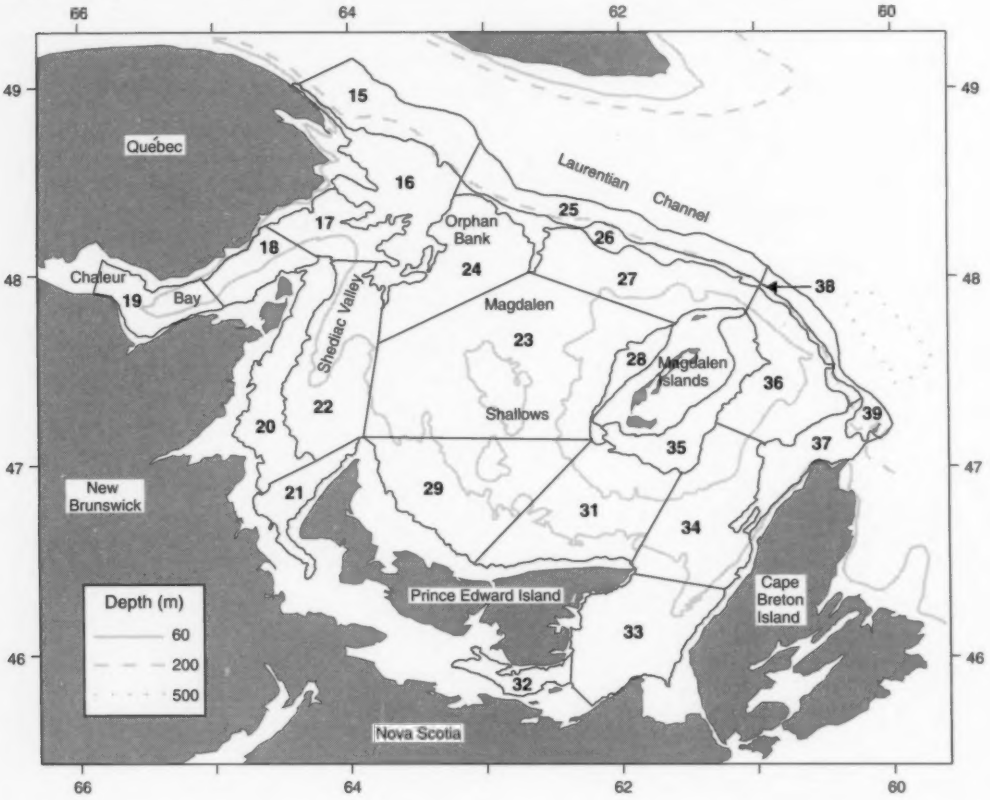
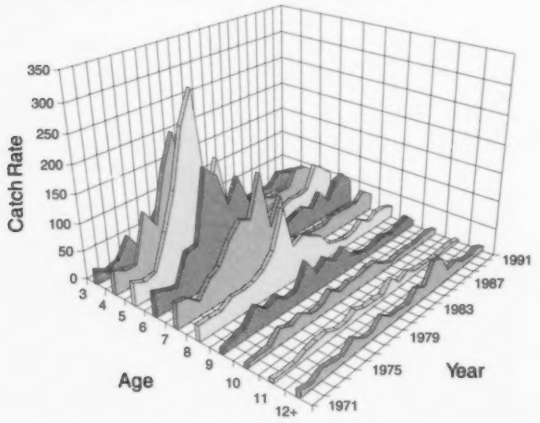


Fig. 2. Relative abundance of American plaice in the southern Gulf of St. Lawrence, 1971–1992. Catch rate is the stratified mean catch per standard tow in the September survey.



has been conducted each September since 1971. Surveys used a stratified random design, with stratification based on depth and geographic region (Fig. 1). Surveys were conducted by the research vessel *E.E. Prince* using a Yankee 36 trawl from 1971 to 1985, by the *Lady Hammond* using a Western IIA trawl from 1986 to 1991, and by the *Alfred Needler* using a Western IIA trawl in 1992. These vessels and trawls are described by Nielsen (1994). Survey procedures are described by Hurlbut and Clay (1990). The target fishing procedure in all years was a 30-min tow at 3.5 knots (1 knot = 1.852 km/h). All catches were adjusted to a standard tow of 1.75 nautical miles (1 nautical mile = 1.852 km). A comparative fishing experiment in 1985 between the *E.E. Prince* and *Lady Hammond* revealed a highly significant difference in fishing efficiency for plaice between these two vessels and gears (Nielsen 1994). Catch rates of plaice by the *Lady Hammond* were 1.8 times those by the *E.E. Prince*; we multiplied plaice catches by the *E.E. Prince* by this factor to account for the change of research vessel and gear in 1986. A comparative fishing experiment in 1992 failed to reveal significant differences in fishing efficiency for plaice between the *Lady Hammond* and the *Alfred Needler*, so no adjustments to plaice catches were required to account for the change in research vessel in 1992 (Nielsen 1994).

We described annual variation in the geographic distribution of plaice in September using the data from these research surveys. Analyses of other demersal fishes have revealed differences between age- or size-classes in distribution (e.g.,

Macpherson and Duarte 1991; Jacobson and Hunter 1993; Swain 1993*a*) and in geographic responses to variation in abundance (Swain and Wade 1993; Marshall and Frank 1994; Swain and Sinclair 1994). Other analyses of plaice in the southern Gulf have revealed differences between the sexes in temperature selection (D.P. Swain, unpublished results). Thus, we analyzed variation in geographic distribution separately for each sex and for each age from 3 to 12+. The sexes were sampled separately on surveys in all years but 1984–1986. Data for these 3 years were omitted from our analyses unless otherwise noted.

Catches in the commercial plaice fishery are not well known because of a high rate of discarding at sea (40% or more; Morin and Sinclair 1993). Thus, we were unable to estimate population size using sequential population analysis. Instead, we used the stratified mean catch per standard tow $\bar{Y}_t$ in the September research surveys as an index of relative abundance (Smith 1988):

$$[1] \quad \bar{Y}_t = \sum_{h=1}^{L} \frac{A_h}{A_T} \bar{y}_{ht}$$

where A_h is the area of stratum h, A_T is the total area surveyed, L is the number of strata, and $\bar{y}_{ht}$ is the mean catch rate of plaice of a particular age and sex in stratum h and year t.

Geographic range

We used the minimum area over which 95% of plaice were *distributed* as a measure of geographic range (Swain and Sinclair 1994). We calculated this area for each age and sex in each year as follows. First, we calculated catch-weighted cumulative distribution functions (cdf) of plaice catch:

$$[2] \quad F(c) = 100 \frac{\sum_{h=1}^{L} \sum_{i=1}^{n_h} \frac{A_h}{n_h} X_{hi} I}{\sum_{h=1}^{L} \sum_{i=1}^{n_h} \frac{A_h}{n_h} X_{hi}} \quad \text{where } I = \begin{cases} 1 & \text{if } X_{hi} \le c \\ 0 & \text{otherwise} \end{cases}$$

where n_h is the number of trawl tows in stratum h, X_{hi} is the number of plaice caught in tow i in stratum h, c is a level of plaice density (i.e., number per standard tow), and other symbols are as described above. $F(c)$ provides an estimate of the percentage of plaice (of a given age and sex in a given year) that occur at a local density of c or less. We also calculated cumulative area in relation to plaice catch:

$$[3] \quad G(c) = \sum_{h=1}^{L} \sum_{i=1}^{n_h} \frac{A_h}{n_h} I \quad \text{where } I = \begin{cases} 1 & \text{if } X_{hi} \le c \\ 0 & \text{otherwise} \end{cases}$$

We evaluated F at intervals of 1.0, and calculated the density c_{05} corresponding to $F = 5$ (Sokal and Rohlf 1981, p. 46). This is the density at or below which the most sparsely distributed 5% of plaice are estimated to occur. $G(c_{05})$ is the estimated area containing the most sparsely distributed 5% of plaice (including areas where no plaice were caught). Thus, the minimum area containing 95% of plaice (D_{95}) is given by

$$[4] \quad D_{95} = A_T - G(c_{05})$$

where A_T is the total survey area. A_T was 70 075 km^2, except in 1978 when only 63 608 km^2 were surveyed and in 1983 and 1988 when 68 945 km^2 were surveyed. For these 3 years when not all strata were sampled, the calculated values of D_{95} were standardized to a total area of 70 075 km^2.

Table 1. Age–sex combinations used to construct NS_{ij}, the abundance index of plaice of sizes similar to age-i (3 to 12+ years), sex-j (male or female) plaice.

	Male		Female	
	Male	Female	Male	Female
3	3,4	3,4	3,4	3,4
4	3,4,5	3,4,5	3,4,5	3,4,5
5	4,5,6	4,5,6	4,5,6	4,5,6
6	5,6,7	5,6	5,6,7,8	5,6,7
7	6,7,8	6,7	7,8,9,10	6,7,8
8	7,8,9	6,7,8	8,9,10,11	7,8,9
9	8,9,10	7,8	10,11,12+	8,9,10
10	9,10,11	8,9	12+	9,10,11
11	10,11,12+	8,9		10,11,12+
12+	11,12+	9		11,12+

Indices of stock area that are based on higher percentiles of plaice density may be of more relevance to catchability in commercial fisheries if fishing effort is restricted to areas of high fish density (Swain and Sinclair 1994). Thus, we also estimated minimum areas over which 90, 75, and 50% of plaice were spread.

Competition is expected to be most intense among similar individuals (e.g., individuals of similar size because diet is size dependent) but may also occur among age- or size-classes of plaice. It is not clear a priori whether the density dependence of stock area for a particular age and sex of plaice is most appropriately tested using an index of abundance of that age and sex alone or using an index that also includes the abundance of other age–sex combinations. Thus, for each age–sex combination, we tested the density dependence of stock area using each of three indices of relative abundance: (*i*) N, the stratified mean catch per tow of that age and sex of plaice, (*ii*) NS, the catch rate of similarly sized plaice (see Table 1), and (*iii*) NT, the catch rate of all plaice aged 3 years and older (irrespective of sex). For example, the stock area of age-6 male plaice was compared with (*i*) the relative abundance of age-6 male plaice, (*ii*) the relative abundance of male plaice aged 5–7 years plus female plaice aged 5–6 years, and (*iii*) the relative abundance of all plaice aged 3 years and older. The range of ages used in NS for each age–sex combination was based on the mean weight-at-age for each sex in the annual surveys (Morin and Sinclair 1993). These tests used Pearson product–moment correlations between the annual indices of stock area and relative abundance.

To assess the significance of apparent differences between sexes and among ages in the density dependence of stock area, we performed ANOVAs including data from all the age–gender combinations. Stock area was the dependent variable and the model included terms for age, sex, relative abundance (either N, NS, or NT), and their interactions. Because N and NS varied over different ranges for different age–sex combinations, relative abundance was standardized to a mean of 0 and standard deviation of 1 within age- and sex-classes for

analyses using these indices. Analyses used the SAS procedure GLM (SAS Institute 1990).

Regional variation in rates of local density change

Following Myers and Stokes (1989), we assumed that the relationship between local density y_{hit} and total population abundance N_t was described by the model:

$$[5] \quad y_{hit} = \alpha_h N_t^{\beta_h}$$

where y_{hit} is the plaice catch (for a particular age and sex) in tow i of stratum h in year t and N_t is the measure of total population abundance in year t (i.e., the stratified mean catch per tow of that age and sex). Estimates of β_h can be obtained using a log-transformation of eq. 5 (e.g., Myers and Stokes 1989). In our case, this required the addition of a constant to the y_{hit} owing to the presence of zero catches. In preliminary analyses, addition of a constant (0.5) appeared to have a substantial effect on estimates of β_h for strata where plaice were rare. Thus, we tested for regional variation in β_h using a generalized linear model (McCullagh and Nelder 1989) that did not require transformation of the y_{hit}:

$$[6] \quad E[y_{hit}] = \mu_{ht} = \exp(a_h + \beta_h \ln N_t)$$

where a_h is the log of α_h in eq. 5. We assumed a Poisson error model:

$$[7] \quad \mathrm{Var}[y_{hit}] = \phi\mu_{ht}$$

where ϕ is a parameter for extra-Poisson variation, expected because organisms typically show a contagious rather than a random spatial pattern (e.g., Pielou 1977). The significance of regional variation in β was tested by analysis of deviance using the GLIM software package (Payne 1986). These tests used the change in scaled deviance between models assuming separate slopes (β_h) in each stratum or a common slope for all strata. We calculated pseudo-R^2 values, the fraction of the total deviance explained by the model, as a measure of model fit (Swartzman et al. 1992).

If habitat utilization by plaice is density dependent, with the population expanding into marginal habitat as abundance increases, then the β_h values are expected to be relatively low (i.e., $\beta < 1$) in optimal habitat and relatively high (i.e., $\beta > 1$) in areas of marginal habitat (Marshall and Frank 1995). To test this prediction, we needed an index of habitat quality, where quality is defined in terms of density-dependent resources. If the IFD holds, then the geographic pattern of forager density will map the distribution of these resources (MacCall 1990). However, at high population sizes, this map may be distorted by the overuse of poor habitats (Gillis and Kramer 1987) and the influence of density-independent factors like temperature (Swain and Kramer 1995). Thus, we assumed that local plaice density at low population sizes gave the best indication of habitat quality in terms of density-dependent resources. For each age–gender combination, we calculated the mean catch rate of plaice in each stratum for each of the 6 years of lowest abundance and used the average of these as an index of habitat quality for each stratum. We compared this index with the estimates of β_h from the previous analysis, predicting a negative relationship between habitat quality and β_h. We conducted this analysis both within age- and sex-classes (excluding 1984–1986 when the sexes were not sampled separately) and combining catches of plaice of both sexes aged 3 years and older (including 1984–1986).

Distribution in periods of low versus high abundance

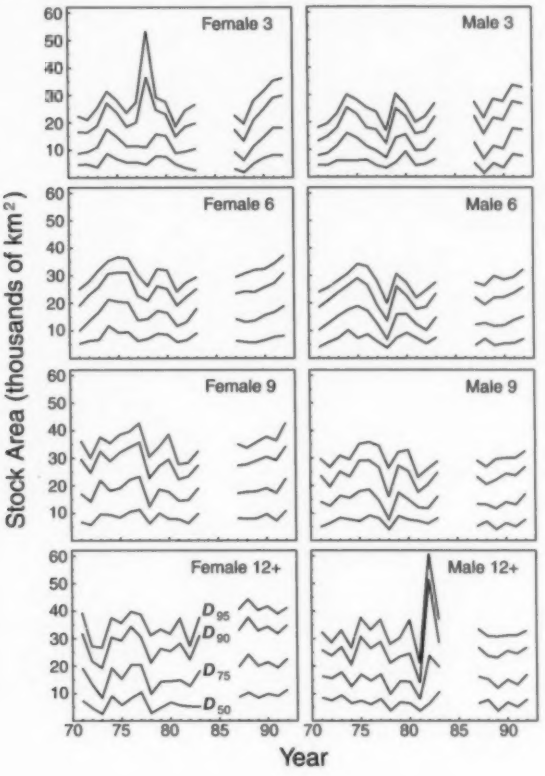
To test for shifts in distribution between periods of low and high abundance, we used a multiplicative (log linear) model comparing the 6 years of lowest abundance and the 6 years of highest abundance for each age–gender combination. The dependent variable was $\ln(y_{hit} + 0.5)$ and the model terms were stratum, abundance period, stratum × abundance period interaction, and year nested within abundance period. The effect of year nested within abundance period was considered a random effect. A separate analysis was performed for each age–gender combination using the GLM procedure of SAS.

To illustrate geographic distribution in periods of low and high abundance, we also mapped plaice distribution for two cases: age-6 males and age-9 females. For each case, we selected the 6 years of lowest abundance and those of highest abundance. Annual mean catch rates of age-6 males varied between 14.5 and 24.5 fish/tow in their low abundance period and 69.5 and 119.3 fish/tow in their high abundance period. Those of age-9 females varied between 4.0 and 8.1 fish/tow in their low abundance period and 19.0 and 38.1 fish/tow in their high abundance period. To combine data from different years in each abundance period, catches were standardized by dividing by the stratified mean catch rate in each year. This standardization also facilitated comparisons between abundance periods, emphasizing changes in distribution rather than changes in overall abundance. Relative variograms were estimated for each abundance period combining data for all 6 years. Contours were drawn using Surfer (Golden Software 1991) after estimating plaice density at points on a 41 × 41 grid by ordinary point kriging. Kriging was done using the Matlab program COKRI (Marcotte 1991). Interpolations used the five nearest samples within the range.

Regional variation in year-class strength

The analyses above treat plaice in the southern Gulf as a single stock. This is the approach that has been taken in the management of these fish (e.g., Morin and Sinclair 1993). Tagging data suggest that there may be two discrete groups of plaice in the southern Gulf, a western group and an eastern group (Powles 1965). If these two groups are different populations, varying independently in abundance, then shifts in distribution in the southern Gulf may simply reflect variation in relative abundance between these two groups. Meristic studies (Powles 1965) and recent genetic analyses (Stott et al. 1992) support the view that plaice in the southern Gulf represent a single population. As a further test of plaice stock structure in the southern Gulf, we tested for independent variation in year-class strength between western (strata 15–29) and eastern (strata 31–39) areas of the southern Gulf. We used a multiplicative model with terms for year-class (1963–1986), age (3–11 years), region (west and east), stratum nested within region, region × year-class interaction, and age × region interaction. The sexes were combined for this analysis, so the years 1984–1986 were included in the analysis. The dependent variable was $\ln(\bar{y}_{jkh} + 0.5)$, where $\bar{y}_{jkh}$ is the mean catch rate of age-j plaice of year-class k in stratum h. Observations were weighted by stratum area.

Fig. 3. Indices of stock area for American plaice in the southern Gulf of St. Lawrence. Indices are the estimated minimum areas containing 50, 75, 90, or 95% of the population (D_{50} – D_{95}, bottom to top lines, respectively).



Results

Geographic range

Variation in plaice stock area showed no strong annual trends (Fig. 3). For younger plaice, stock area tended to increase from low values in 1971 to high values in the mid-1970s and from low or intermediate values in 1988 to high levels in 1991 and 1992. For older females (especially age 12⁺), stock area tended to be high from 1987 to 1992.

Correlations between indices of stock area and relative abundance were, with few exceptions, not significant (Fig. 4). Except for the positive correlations between NT and the geographic ranges of males aged 9 and 10, there was no tendency for range (D_{95}) to expand with plaice abundance. In fact, correlations between range and N or NS tended to be negative for older females, approaching or exceeding significance ($P < 0.05$) for ages 9–11. Correlations with D_{50} tended to be more positive than those with D_{95}. For males, the correlations with D_{50} showed the same pattern for all three abundance indices, increasing from values near zero for age 3 to values approaching or exceeding significance for ages 8 to 10 or 11. However, the number of significant correlations (7) in Fig. 4 is about the number expected by chance alone (6). Correlations with D_{90} and D_{75} showed patterns intermediate to those shown for D_{95} and D_{50} in Fig. 4.

Table 2. Effects of sex, age, and relative abundance on stock area (D_{95} or D_{50}).

	D_{95}		D_{50}	
	N	NT	N	NT
Model R^2	0.427	0.403	0.273	0.290
% model R^2				
Sex	26.9****	28.5****	22.2****	20.9****
Age	57.7****	61.1****	49.5****	46.6****
Abundance	1.0	1.4	5.2**	15.9****
Sex × age	4.8	5.1	9.7	9.1
Abundance × sex	0.1	0.1	2.5	0.0
Abundance × age	3.4	2.2	6.2	5.9
Abundance × sex × age	6.0	1.6	4.6	1.6
β	–3.90	0.0173	2.47	0.0172
SE	2.44	0.0094	0.96	0.0036

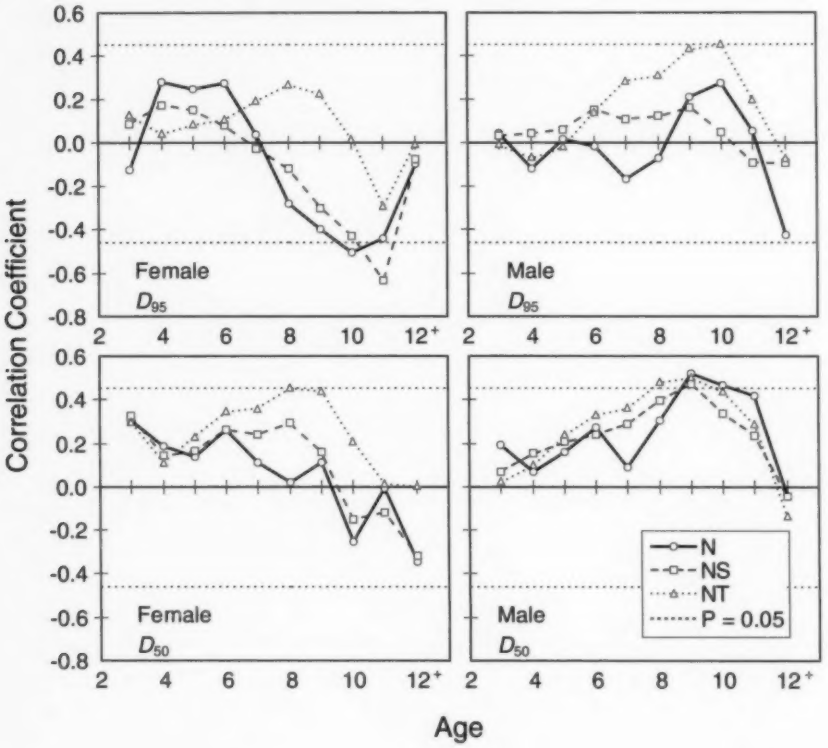
Note: Abundance is either N, the abundance of each age–sex class, or NT, total age-3⁺ abundance. The percentage of the model R^2 contributed by each model term is shown. Asterisks indicate significance level (** $P = 0.01$; **** $P < 0.0001$). β is covariate (relative abundance) slope, estimated using a model without interaction terms (because these are all nonsignificant). Sample size is 380.

Models including age, sex, abundance, and their interactions explained about 40% of the variation in D_{95} and about 30% of the variation in D_{50} (Table 2). Effects of age and sex were highly significant, accounting for most of the explained variation in stock area. Stock areas were greater (i.e., distribution was more spread out) for females than for males and for older plaice (Fig. 5). D_{50} was density dependent but D_{95} was not (Table 2). For D_{95}, the relationship with abundance was not even in the predicted (positive) direction using N (or NS). For D_{50}, although density dependence was significant and in the predicted direction, it contributed only a relatively small percentage of the model R^2. None of the interaction terms was significant, indicating that the apparent differences between sexes and among ages in the density dependence of stock area (suggested by the patterns in Fig. 4) were not significant. Results using NS (not shown in Table 2) were similar to those using N. Results for D_{90} and D_{75} (not shown in Table 2) were intermediate between those for D_{95} and D_{50}, though generally more similar to results for D_{95}.

Regional variation in rates of local density change

The rate of local density change (i.e., β_h) differed significantly among strata in all cases except age-12⁺ males (Table 3). Although statistically significant, regional variation in β_h explained only a small percentage of the total deviance in plaice catch rates. The model with terms for stratum, relative abundance, and their interaction accounted for 36–60% of the deviance in plaice catch rates, but the interaction term (which accounted for heterogeneity in β among strata) contributed only 2–6% of the model R^2 (Table 3). Contrary to predictions, there was no tendency for β to be negatively correlated with habitat quality (as inferred from local density at low population sizes) (Table 3; Fig. 6). None of the correlations between β and the index of habitat quality were significant, and 13 of the 21 correlations were positive.

Fig. 4. Correlations between indices of stock area (D_{50} or D_{95}) and relative abundance (N, NS, or NT) for American plaice in the southern Gulf of St. Lawrence. D_{50} and D_{95} are defined in Fig. 3. N is the abundance index for plaice of a particular age–sex class, NS is the abundance index for plaice that are of a size similar to that age–sex class (see Table 1), and NT is the abundance index for all plaice aged 3 years and older (see text for details). Correlation coefficients between the broken lines are not significant at the 0.05 level.



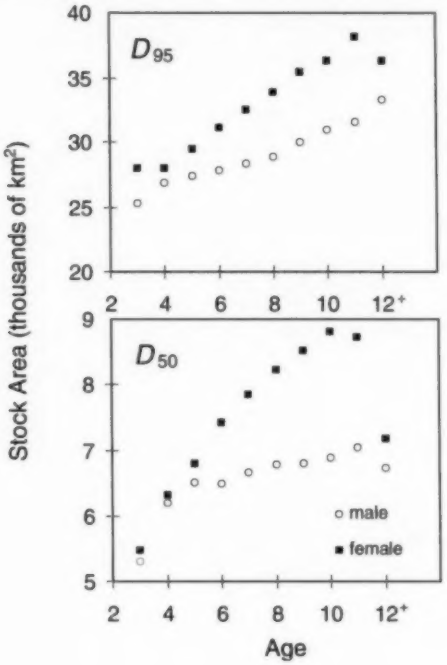
Estimates of β varied widely among strata with mean catch rates near zero (Fig. 6). For some of these strata, β was estimated to be very high for one age-class and very low for another (e.g., compare stratum 32 between age-6 and age-9 males in Fig. 6). This wide variation in estimates of β for strata with low catch rates of plaice could reflect chance fluctuations in densities near zero and might explain our failure to observe the predicted negative correlation between β and local density. We repeated this analysis excluding strata where habitat appeared to be mostly unsuitable for plaice. In September, plaice in the southern Gulf strongly prefer intermediate depths and cold temperatures (D.P. Swain and R. Morin, unpublished results). We excluded the shallow inshore strata (20, 21, 28, 32, 35) and deep offshore strata (15, 25, 39) with warm bottom temperatures (Swain 1993*b*). This reduced the model R^2 slightly to 24–53%. Regional variation in β remained significant ($P < 0.01$) for all cases except age-12$^+$ males but accounted for only 3–10% of the model R^2. There was again no tendency for β to be negatively correlated with local density (Table 3). Thirteen of the 21 correlations were positive and no correlation was significant except for the negative correlation for age-3 males ($P = 0.0043$). Considering the number of correlations tested (21), even the latter correlation was not significant according to a Bonferroni test (Rice 1989).

Distribution in periods of low versus high abundance

No large shifts in the distribution of plaice among strata occurred between periods of low and high abundance (Fig. 7). Average catch rates were high in the same strata in both abundance periods. Mean catch rates by stratum did tend to be greater fractions of the maximum stratum mean in the high than in the low abundance period (Fig. 7), indicating a more even distribution of plaice among strata in the high abundance period. However, although these differences in geographic distribution between abundance periods were statistically significant in most cases, they accounted for only a small proportion of the total variation in catch rates (Table 4). Models with terms for stratum, abundance period, their interaction, and year nested within abundance period accounted for 43–62% of the variation in log-transformed catch rates. The interaction term was significant in 19 of 20 cases, but the amount of variation explained by this term was only 3–9% of the model R^2.

Likewise, no striking differences in distribution are apparent between periods of low and high abundance in the two

Fig. 5. Age- and sex-dependent stock area of American plaice in the southern Gulf of St. Lawrence. Stock areas are the means for 1971–1992 (excluding 1984–1986). D_{50} and D_{95} are defined in Fig. 3.



cases illustrated in Fig. 8. In both cases, plaice are most concentrated in the same three general areas during periods of both low and high abundance: (*i*) Chaleur Bay, (*ii*) the Shediac Valley and central Magdalen Shallows, and (*iii*) the area between Prince Edward and Cape Breton islands. There is no indication of range expansion with increasing abundance in either case.

Regional variation in year-class strength

Variation in estimated year-class strength was closely parallel between the western and eastern regions of the southern Gulf (Fig. 9). The multiplicative model with terms for year-class, age, region (east versus west), stratum, year-class × region interaction, and age × region interaction explained 79% of the variation in mean catch rates. All terms were highly significant ($P < 0.0005$) except for the year-class × region interaction ($P = 0.41$). This interaction term contributed only 0.15% of the model R^2. These results support the view that plaice in the southern Gulf represent a single population (or that year-class strength has varied synchronously between stock components). Thus, variation in relative abundance between regional subpopulations does not appear to contribute to or obscure annual variation in the geographic distribution of plaice in the southern Gulf.

Discussion

The factors with the strongest effects on plaice stock area were age and gender. Stock area tended to be greater for older plaice and for females. For older plaice, this could reflect more widely distributed prey resources or stronger competitive interactions for larger individuals. However, differences between the sexes in size or abundance do not appear to account for the effect of gender on stock area. For example, age-6 females are smaller than age-7 males (Morin and Sinclair 1993) but have greater stock areas. Likewise, for ages 3–9, females tend to be less abundant than males (R. Morin, unpublished results), but have greater stock areas. The greater size-at-age of females suggests that the fitness benefits associated with growth are greater for females than for males. Perhaps their greater stock areas reflect more intensive foraging activity and competitive interactions among females.

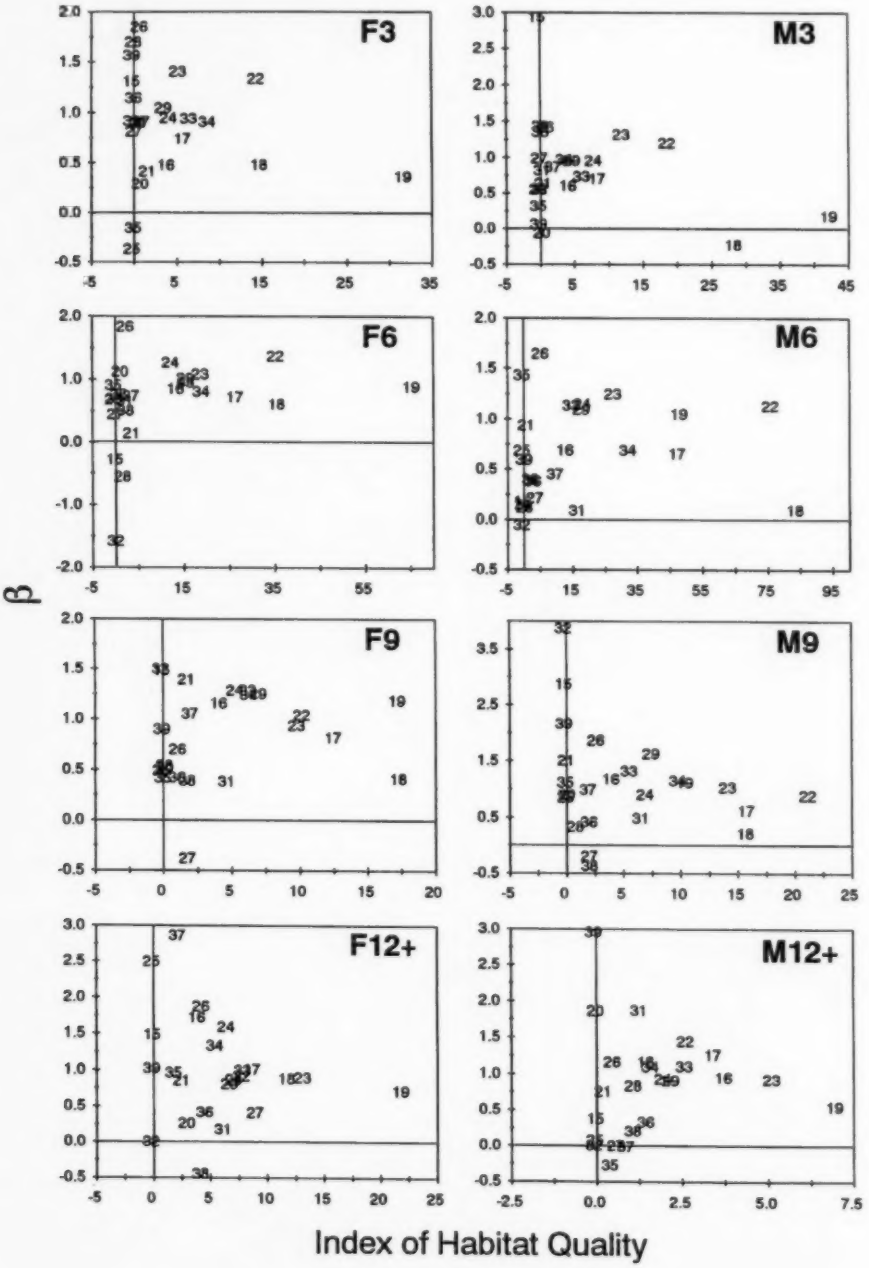
Theoretical models predict the expansion of population range into marginal habitat as population size increases and competition reduces the suitability of optimal habitat (e.g., MacCall 1990). Consequently, percent changes in local density with changes in population size should be greater in marginal than in optimal habitat (e.g., Marshall and Frank 1995). However, for American plaice in the southern Gulf of St. Lawrence, we observed no tendency for population range to increase with population size, despite fivefold variation in

Table 3. Tests for regional variation in rates of local density change with changes in population size.

Sex	Age	R^2	$\%R^2$	r	r_{sub}
Male	3	0.415	5.4^{****}	–0.290	-0.672^{**}
	4	0.524	4.7^{****}	0.019	–0.078
	5	0.580	3.1^{****}	0.238	0.231
	6	0.580	3.9^{****}	0.128	–0.009
	7	0.571	5.5^{****}	0.187	0.236
	8	0.537	5.4^{****}	0.069	0.110
	9	0.463	3.0^{**}	–0.272	0.062
	10	0.434	5.4^{****}	–0.154	–0.064
	11	0.394	3.8^{**}	–0.114	–0.004
	12⁺	0.359	3.2	0.048	0.105
Female	3	0.448	3.9^{***}	–0.145	–0.435
	4	0.519	4.2^{****}	–0.157	–0.237
	5	0.602	2.6^{****}	0.254	0.160
	6	0.586	2.4^{***}	0.288	0.079
	7	0.589	3.3^{****}	0.299	0.174
	8	0.573	4.4^{****}	0.333	0.251
	9	0.569	2.9^{****}	0.129	0.255
	10	0.532	3.1^{****}	0.170	0.243
	11	0.513	2.4^{**}	–0.061	0.026
	12⁺	0.369	5.8^{***}	–0.209	–0.274
Both	3⁺	0.556	4.1^{****}	0.071	0.009

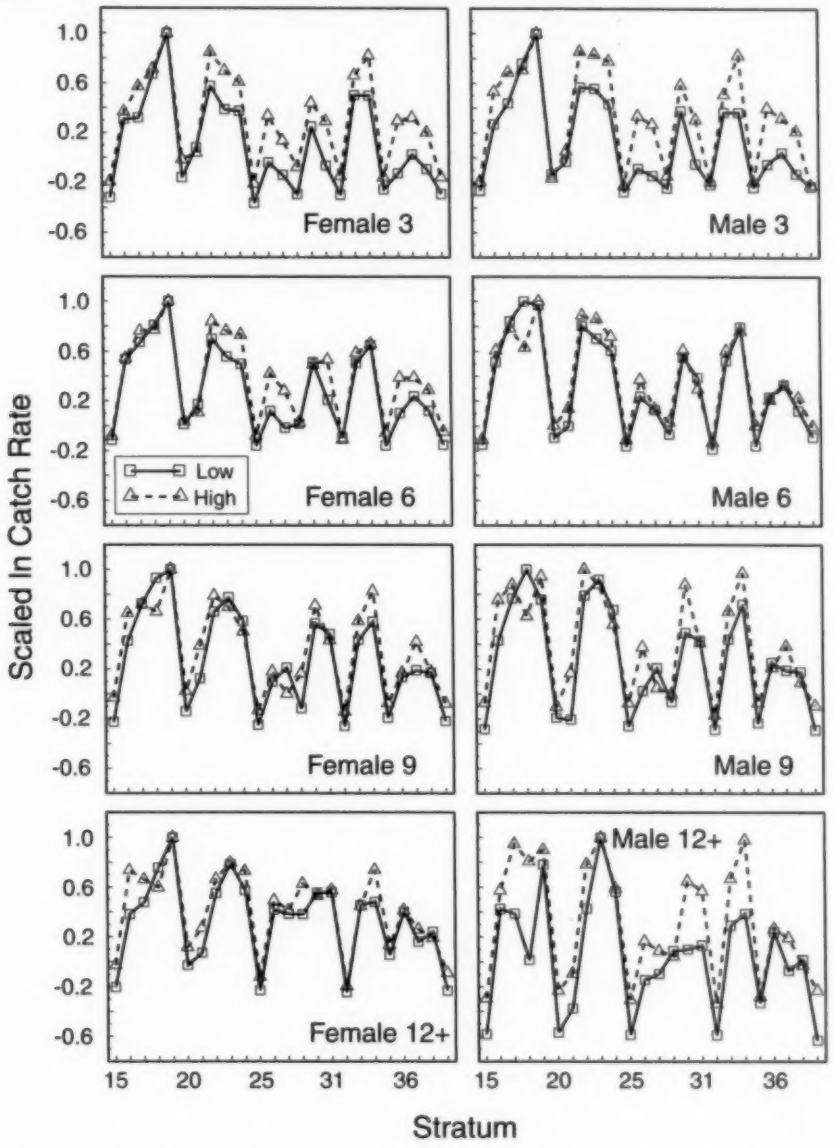
Note: R^2 is the fraction of the total deviance in plaice catch rates explained by a model with stratum as a factor, ln-transformed stratified mean catch per tow as a covariate, and a term testing for heterogeneity of covariate slope β among strata. $\%R^2$ is the percentage of the model R^2 accounted for by the latter term. r is the correlation between β for stratum j and the index of habitat quality for that stratum (i.e., the mean catch rate in that stratum for the 6 years of lowest abundance). r_{sub} is the correlation omitting strata rarely occupied by plaice (strata 15, 20, 21, 25, 28, 32, 35, 39). Asterisks indicate significance level ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). Sample size is 1735 (or 2027 for age 3⁺, sexes combined) for the GLIM analysis, 24 for r, and 16 for r_{sub}.

Fig. 6. Relationship between habitat quality and rate of local density change with changes in population size. The index of habitat quality is the local density at low population size, obtained from the mean catch rate in a stratum during the 6 years of lowest abundance. β is from eq. 6 in the text. The plotted symbols are stratum numbers (see Fig. 1).



the abundance of plaice. Likewise, we detected no tendency for percent changes in plaice density to be greater in marginal habitat than in optimal habitat as population size changed (where habitat quality was assigned on the basis of local plaice density at low population sizes). Finally, although geographic distribution differed somewhat between periods of low and

Fig. 7. Average catch rates of American plaice by stratum during the 6 years of lowest and highest abundance. Average catch rates are scaled by the maximum average in each abundance period to emphasize any difference in distribution between abundance periods.

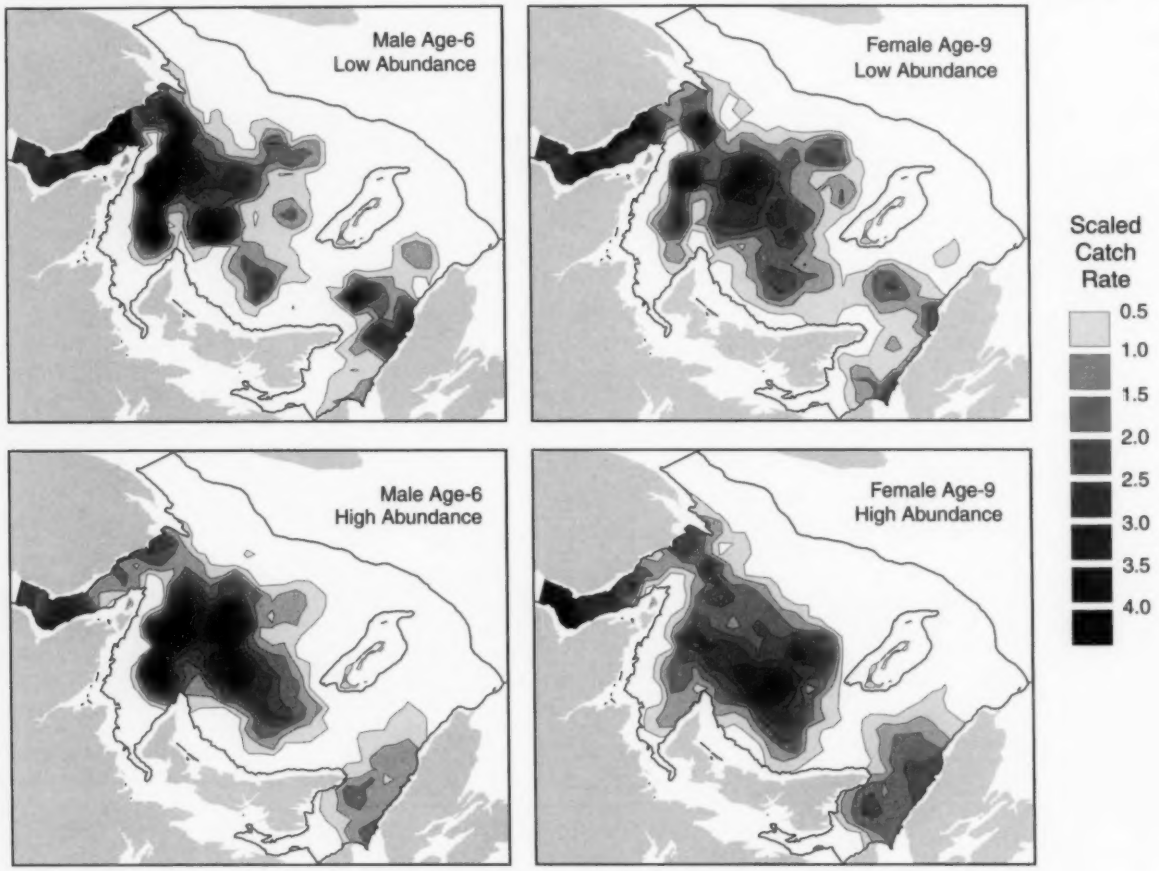


high abundance, we found no substantial shifts in distribution between the two abundance periods.

These results for plaice are in striking contrast to those for cod in the southern Gulf (Swain 1993*a*; Swain and Wade 1993; Swain and Sinclair 1994). Population range increased with cod abundance (Swain and Sinclair 1994). As population size increased, local density increased relatively slowly in the areas where cod were concentrated at low population sizes and relatively rapidly in surrounding areas (Swain and Wade 1993). For older cod, this reflected not only an expansion of range, but also a shift in distribution, with peak densities shifting from the regions preferred at low abundance to adjoining regions at high abundance (Swain and Wade 1993; Swain 1993*a*).

Differences between cod and plaice in environmental tolerances may contribute to the differences between the two species in their geographic responses to variation in abundance. Density-dependent temperature selection provides a possible explanation for the shift in cod distribution between periods of low and high abundance in the southern Gulf. Cod prefer relatively warm temperatures when ration levels are high (e.g., Jobling 1988) but may select relatively cold temperatures in

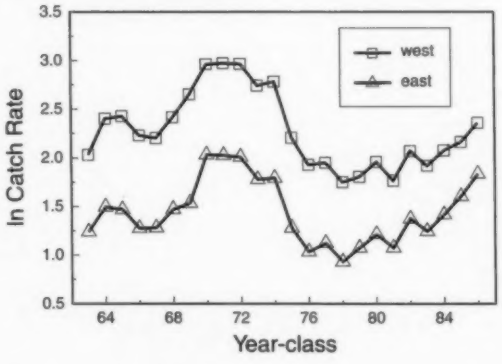
Fig. 8. September distribution in the southern Gulf of St. Lawrence for two selected age- and sex-classes of American plaice. Distribution is shown for the 6 years of lowest abundance and the 6 years of highest abundance. Note that catch rates are standardized to the same average level (1.0) in both abundance periods to emphasize changes in distribution rather than changes in overall abundance.



the wild where ration levels may be low (e.g., Swain and Kramer 1995). Swain and Kramer (1995) suggested that cod distribution in the southern Gulf shifts from warm (high cost), shallow (high resource) areas when abundance is low to colder, deeper areas when abundance is high to reduce metabolic costs as food rations decline. Plaice also tend to select somewhat colder temperatures when rations are reduced (Morgan 1993). However, in contrast to cod, they prefer cold temperatures even when fed high rations (Morgan 1993). Thus, large shifts in distribution owing to density-dependent temperature selection are not expected for plaice.

Restricted temperature preferences may explain the absence of large density-dependent shifts in plaice distribution, but they do not explain the failure of plaice range to expand with increasing abundance. One possible explanation for this apparent density independence of plaice range in the southern Gulf is that variation in plaice abundance has been insufficient to cause range expansion or contraction. Catch rates of plaice in the annual bottom-trawl survey indicate that plaice abundance in the southern Gulf has varied fivefold since 1971. Catch rates of cod in this survey suggest somewhat wider

Fig. 9. Year-class strengths of American plaice in the western (strata 15–29) and eastern (strata 31–39) regions of the southern Gulf of St. Lawrence.



variation in cod abundance. However, catchability of cod to the survey appears to be density dependent (Swain et al. 1994),

possibly as a result of the density-dependent shifts in cod distribution (Swain 1993*a*; Swain and Kramer 1995). Thus, catch rates in the survey appear to overestimate variation in cod abundance. Sequential population analysis suggests that cod abundance has varied about fourfold in the southern Gulf since 1971 (Sinclair et al. 1994). Thus, fourfold variation in abundance has resulted in range expansion in the case of cod but not in the case of plaice.

The relationship between cod abundance and geographic range appeared to be asymptotic in the southern Gulf, at least for older cod (Swain and Sinclair 1994). Once cod range had expanded to about 60% of the survey area, all suitable habitat appeared to be occupied and there was no additional range expansion with further increases in cod abundance. For older cod, most suitable habitat appeared to be occupied by about the median level of abundance since 1971 (see Fig. 7 in Swain and Sinclair 1994). Perhaps plaice geographic range has been independent of abundance in the southern Gulf because abundance has not fallen below the level at which all suitable habitat is occupied. The minimum stratified mean catch rate of plaice in the annual survey (128 age-1$^+$ plaice per standard tow, the mean catch by the *E.E. Prince* in 1984 unadjusted for vessel changes; Morin and Sinclair 1993) is several times that of cod (26 age-1$^+$ cod per standard tow, the unadjusted mean catch by the *E.E. Prince* in 1975; Sinclair et al. 1994). This suggests that the minimum levels of plaice abundance in the southern Gulf are much greater than the minimum levels of cod abundance, unless catchability to the survey is much greater for plaice than for cod. Similarly, the minimum average catch rate of plaice in the southern Gulf survey is comparable to or greater than the maximum average catch rates of plaice in surveys of regions of the Scotian Shelf (Simon and Comeau 1994) and the Northeast Newfoundland and Labrador Shelf (Brodie et al. 1993), again suggesting that plaice abundance in the southern Gulf has not fallen to very low levels in the 1971–1992 period. Given the narrower temperature preferences of plaice than cod, suitable habitat may also be more limited for plaice than for cod. This is consistent with the possibility that all suitable habitat may be occupied by plaice at the levels of abundance seen since 1971.

An alternative possibility is that plaice abundance in the southern Gulf has never reached levels high enough for competition for density-dependent resources to occur. We believe that this alternative explanation is unlikely given the very high average catch rates in the abundance surveys for some years (i.e., unadjusted averages of 295–581 fish per standard tow in 1974–1981).

When abundance is sufficiently high that all suitable habitats are occupied, some habitat selection models predict that the proportion of the population in each habitat will be independent of population size (e.g., the input-matching rule, Parker 1978). However, if the strength of interference increases with forager density, then the proportion of foragers occupying poorer patches should continue to increase with population size (Sutherland 1983). In a laboratory study, Gillis and Kramer (1987) found that the distribution of foragers among patches did depend on population size, with the proportion of fish occupying poorer patches increasing with the total number of fish. The slight tendencies for the area containing 50% of plaice (D_{50}) to expand as abundance increased and for plaice to be more evenly distributed among strata at high abundance are consistent with the prediction that the proportion of foragers occupying poorer patches should increase with population size. These results contrast with our failure to observe a negative correlation between β and an index of habitat quality. This latter result indicates that there was no tendency for the proportion of plaice occupying poorer strata to increase with population size.

Table 4. Tests for differences in geographic distribution between periods of low and high plaice abundance.

	Male		Female	
Age	R^2	$\%R^2$	R^2	$\%R^2$
3	0.478	4.4*	0.506	4.8**
4	0.571	3.6**	0.552	4.3**
5	0.582	4.0**	0.589	3.3**
6	0.585	3.9***	0.621	4.1****
7	0.566	4.7****	0.610	3.0***
8	0.543	6.6****	0.602	3.9****
9	0.532	6.7****	0.610	6.1****
10	0.489	4.4*	0.574	7.9****
11	0.431	3.6	0.535	7.7****
12$^+$	0.435	9.3****	0.454	3.8*

Note: R^2 is for the multiplicative model with abundance period, stratum, and their interaction as fixed effects and year nested within abundance period as a random effect. $\%R^2$ is the type III sum of squares (SS) for the interaction term as a percentage of the model SS. Asterisks indicate the significance level of the interaction term (significance levels as in Table 3). The two abundance periods are the 6 years of lowest abundance and the 6 years of highest abundance for the age–sex class tested. Sample size varies between 921 (age-5 males) and 1252 (age-8 males), depending on the years included in these two abundance periods.

We can suggest a number of factors that may contribute to our failure to observe the predicted negative correlation between β and habitat quality. First, our characterization of habitat quality may be incorrect. We have no direct estimates of the distribution of prey resources. Thus, we assumed that the geographic pattern of plaice density mapped the distribution of these density-dependent resources. We used plaice density during years of low abundance to map these resources because of the possible effects of density-independent costs on fish distribution at high abundance (Swain and Wade 1993; Swain and Kramer 1995). However, the correspondence between forager and resource distributions may also be distorted at low population sizes. For example, overuse of poor patches is predicted if foragers differ in competitive abilities (Houston and McNamara 1988). Our approach also assumes that habitat quality is stable over time at the geographic scale of strata. Annual shifts in the distribution of prey and thus of habitat quality may explain our failure to observe a negative correlation between β and our index of habitat quality. However, because diet is size dependent in plaice (Powles 1965; J.M. Hanson, Department of Fisheries and Oceans, P.O. Box 5030, Moncton, NB E1C 9B6, Canada, unpublished results), parallel changes in the distribution of a wide variety of prey taxa would have to be postulated to explain our failure to observe the predicted correlation for all ages of plaice. Second, it may be important to consider interactions between the effects of density-dependent resources and density-independent factors like temperature (e.g., Swain and Kramer 1995). Third, effects of

interspecific competition between plaice and other species (e.g., cod) need to be considered. Finally, the importance of scale and environmental patchiness needs to be examined. Theoretical models and laboratory experiments have examined distribution among homogeneous habitats or patches. Large-scale population-level studies like this one examine distribution among geographic regions or strata. Each stratum or region is likely to be a mosaic of habitats. This difference may contribute to apparent discrepancies between model predictions and empirical results at large geographic scales.

The idea that population range expands and contracts with population abundance is becoming well established in the fisheries literature (e.g., Winters and Wheeler 1985; Crecco and Overholtz 1990; but see Marshall and Frank 1994). The present study presents an exception to this rule over a fivefold variation in population size. Further research, both theoretical and empirical, is needed to determine the generality of this rule and the conditions under which it does not apply. This research is important for fisheries management and stock assessment because of the relationship between population range and catchability (e.g., Paloheimo and Dickie 1964).

Acknowledgements

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Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids

Dianne B. Morris, Kenny R. Richard, and Jonathan M. Wright

Abstract: We have cloned 76 microsatellite sequences from rainbow trout (*Oncorhynchus mykiss*), each consisting of tandemly repeated arrays of either $(GT)_n$ or $(GA)_n$. To summarize patterns of genetic structure, each microsatellite was characterized according to the length and complexity of the repetitive sequences. Two microsatellites were developed for use as genetic markers in *O. mykiss*. The locus PuPuPy contained >100 repeats of a trinucleotide motif of the general form purine-purine-pyrimidine. The locus Omy77 contained ~18–40 repeats of the dinucleotide GA. Length polymorphisms were identified through polymerase chain reaction (PCR) analysis, and each locus displayed at least 8–9 alleles. Primers designed from the *O. mykiss* sequence were employed to PCR-amplify and clone fragments from *Oncorhynchus clarki*, *Oncorhynchus keta*, *Oncorhynchus kisutch*, *Oncorhynchus tshawytscha*, *Salmo salar*, and *Salvelinus fontinalis*. The Omy77 primers failed to amplify *Salvelinus fontinalis* DNA. Sequencing of clones confirmed that the PCR-amplified fragments were derived from homologous loci. Both loci appear to be polymorphic in most species examined, suggesting that microsatellite markers isolated from one species of salmonid are likely to also be useful in genetic studies of other salmonid species.

Résumé : Nous avons cloné 76 séquences microsatellites du saumon arc-en-ciel (*Oncorhynchus mykiss*), chacune constituée de motifs $(GT)_n$ ou $(GA)_n$ se répétant en tandem. Pour avoir une idée de la composition génétique de ces fragments, on a caractérisé chaque microsatellite en fonction de la longueur et de la complexité des séquences répétitives. Deux microsatellites ont été conçus pour être utilisés comme marqueurs génétiques chez *O. mykiss*. Le locus PuPuPy renfermait plus de 100 répétitions d'un motif trinucléotidique de forme générale purine-purine-pyrimidine. Le locus Omy77 renfermait environ 18–40 répétitions du dinucléotide GA. Les polymorphismes de la taille des fragments ont été repérés par amplification à la PCR, et chaque locus comportait au moins 8–9 allèles. Les amorces obtenues de la séquence provenant de *O. mykiss* ont été utilisées pour amplifier par la PCR et cloner les fragments provenant de *Oncorhynchus clarki*, de *Oncorhynchus keta*, de *Oncorhynchus kisutch*, de *Oncorhynchus tshawytscha*, de *Salmo salar* et de *Salvelinus fontinalis*. L'ADN de *Salvelinus fontinalis* n'a pu être amplifié par les amorces de Omy77. Le séquençage des clones a confirmé que les fragments amplifiés par la PCR provenaient de locus homogènes. Les deux locus sont polymorphes chez la plupart des espèces examinées, ce qui indique que les marqueurs microsatellites isolés d'une espèce de salmonidé devraient probablement aussi être utiles dans les études génétiques d'autres salmonidés.
[Traduit par la Rédaction]

Introduction

Microsatellite DNA sequences consist of tandem arrays of a small sequence motif, often $(GT)_n$ or $(GA)_n$ (reviewed in Wright 1993, 1994). They generally appear to be noncoding and selectively neutral, and are frequently polymorphic as a result of allelic variation in the number of repeats in the tandem array (Queller et al. 1993). Such characteristics offer great potential for use as genetic markers (Wright and Bentzen 1994). Moreover, with current molecular methods it is feasible to score microsatellite length polymorphisms in large numbers of individuals for genetic analyses within and between populations. This is made possible by two features of microsatellites, namely that the tandem arrays have a maximum length of only a few hundred base pairs and that the repetitive sequences are usually flanked at each end by unique DNA. The unique sequences allow specific microsatellite loci to be targeted for polymerase chain reaction (PCR) amplification (Saiki et al. 1988), while the relatively short length of the array facilitates size fractionation and precise scoring of alleles on polyacrylamide sequencing gels.

Although microsatellites have already proven to be powerful single locus markers for a variety of genetic studies (Queller et al. 1993), the development of species-specific primers for PCR amplification of alleles can be expensive and

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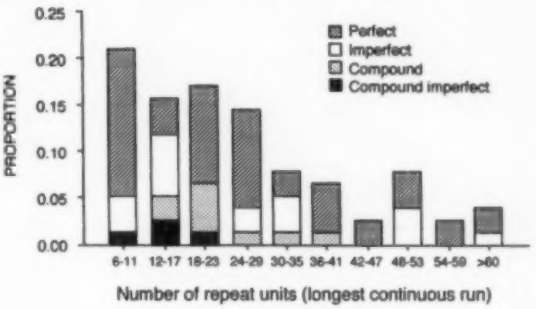
time consuming. However, previous work has shown that some microsatellite loci are conserved within related taxa so that primers developed to amplify markers in one species may amplify the homologous markers in related species as well (e.g., Moore et al. 1991; Schlötterer et al. 1991; Brooker et al. 1994).

In this study, we have cloned and sequenced several microsatellite loci from rainbow trout (*Oncorhynchus mykiss*). We have summarized the overall pattern of microsatellite structure in this one species by categorizing sequences according to the complexity and length of the repetitive array. Two sequences were also subjected to detailed analysis to evaluate their use as genetic markers. Thus, we designed PCR primers to amplify these microsatellites from DNA of rainbow trout and several other salmonids. Genetic variation at the PCR-amplified markers was examined in two different ways. First, the markers were tested for allelic length variation by size fractionation of PCR products; both markers appear to be polymorphic in most of the seven salmonid species examined. Second, PCR-amplified fragments were cloned from one individual of each species. Sequencing of stretches of DNA flanking either side of the repetitive array in each clone confirmed that both microsatellite loci were conserved between species, although interspecific sequence comparisons failed to prove useful for clarifying the phylogenetic placement of the salmonid species.

Fig. 1. Proportion of different size-classes of 76 microsatellite sequences isolated from rainbow trout. Categories of perfect, imperfect, compound, and compound imperfect are defined according to Weber (1990). Perfect repeats consist of a continuous, uninterrupted run of the dinucleotide repeat unit. In imperfect repeats, the run of repeat units is interrupted by 1–3 intervening bases of unique sequence. In compound repeats, the main (i.e., longest) repetitive array is adjacent to another repetitive array containing a different repeat unit. As in Weber (1990) for imperfect and compound repeats, the size of each microsatellite was counted as the length of the longest continuous stretch of repeats (e.g., $(GT)_{30}A(GT)_{17}$ would be categorized as an imperfect microsatellite of size 30).



Methods and materials

Isolation and sequencing of microsatellites

Oncorhynchus mykiss microsatellites were cloned and sequenced from two size-selected genomic libraries constructed using standard techniques described by Brooker et al. (1994) and Wright and Bentzen (1994). All clones were screened using a $(GT)_{15}$ oligonucleotide probe. Some were also screened with $(GA)_{15}$. Each microsatellite sequence was classified according to size and complexity (see Fig. 1 caption), following the definitions of Weber (1990), and as also employed by Brooker et al. (1994).

PCR primers for two of the microsatellites

Among several polymorphic microsatellites identified in *O. mykiss*, two were analyzed in further detail. The first locus (PuPuPy) consisted of a tandem array of a trinucleotide motif, purine-purine-pyrimidine (predominantly GAC/T). Unlike all of the other microsatellites isolated, PuPuPy was isolated serendipitously in the course of sequencing a genomic library for other purposes. The second locus, Omy77, consisted of a GA dinucleotide repetitive array and was identified by cloning efforts directed towards isolating microsatellites.

Primers for PCR amplification of the PuPuPy and Omy77 microsatellites were designed to target unique sequence immediately adjacent to the repetitive array (primer sites underlined in Fig. 2). However, for cloning of microsatellites in different species, primers were redesigned to target sites that were farther away from the actual repetitive array so as to maximize the amount of flanking sequence incorporated in the PCR products (cf. Fig. 2). As well, to facilitate cloning, a restriction site (*Eco*RI) was included on the 5′ end of each PCR primer (e.g., the forward primer of Omy77, including the *Eco*RI linker, was ATCACCGGGAATTCCGTTCTCTACTGAGTCAT).

DNA samples

Genomic DNA samples from several hundred hatchery individuals of *O. mykiss* (cf. Herbinger et al. 1996) were generously supplied by C. Herbinger, Marine Gene Probe Laboratory (MGPL). DNA from other salmonid species (2–6 individuals each) were available from the MGPL genomic DNA bank. Sampled species included *Oncorhynchus clarki* (cutthroat trout), *Oncorhynchus keta* (chum salmon), *Oncorhynchus kisutch* (coho salmon), *Oncorhynchus tshawytscha* (chinook salmon), *Salmo salar* (Atlantic salmon; two each from Nova Scotia and from Ireland), and *Salvelinus fontinalis* (brook trout).

PCR amplifications for scoring allelic length variation

Typical PCR reactions contained 10 ng of genomic DNA, 0.05 μM ^{32}P-labelled reverse primer, 0.45 μM unlabelled reverse primer, 0.5 μM unlabelled forward primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM $MgCl_2$, 0.001% gelatin, 0.2 mM deoxynucleoside triphosphates (dATP, dGTP, dCTP, dTTP), and 0.5 U of *Taq* DNA polymerase (prepared by the method of Engelke et al. 1990) in a total reaction volume of 10 μL. The reverse PCR primer was radiolabelled with [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) and T4 polynucleotide kinase (Pharmacia).

PCR amplification typically consisted of 30 cycles of denaturation at 94°C for 30–60 s, annealing at 52°C for 30 s, and extension at 72°C for 1 s. Following amplification, 10 μL of stop dye (Pharmacia) was added to each sample and the products were denatured at 80°C for 5 min.

PCR products were size fractionated at 2100 V in

Fig. 2. Nucleotide sequences of microsatellite loci amplified by PCR from salmonid DNA. Sequences are aligned with respect to the *O. mykiss* sequence. Dots indicate identities and dashes denote insertions or deletions. The asterisks outline the microsatellite repetitive array. The primer sites used in the amplification of DNA for cloning are shown at either end of the sequences and are not included in the alignment. The underlined sequences indicate the primer sites originally used to score allelic length polymorphisms in *O. mykiss*.

a) Microsatellite locus PuPuPy

```
mykiss       CTACTCTGCACCTTCCAGACCCCTCTTGCCCCTGGCGTCAAGGCTCAAGACCTGCCGCTGGCTCCCCAACAGGAT
keta                                                                ..............-C.............
tshawytscha                                                         ....-..........................C.............
kisutch                                                             ..............................C.............
clarki                                                              ..............................C.............
salar-NS                                                            ............................A....TC.............
salar-IRE                                                           ............................A....TC.............
fontinalis                                                          ............................A.....C.A...........

                                                                                    *********
mykiss       GTAGTAGCTGATGACGAGGGGGAGCATGCAGCGGATGTAGGGGGAGAT(PuPuPy)nCACACAGGTAAGTTACGT
keta         ..................................G.............                      ....................
tshawytscha  ..................................G.............                      ....................
kisutch      ..................................G.............                      ....................
clarki       ..................................G.............                      ....................
salar-NS     ..................................G.............                      ....................
salar-IRE    ..................................G.............                      ....................
fontinalis   ................G..................G.............                      ....................

mykiss       CTTTTCACTTAAATGATGCTGCATTCTTTATCCACCTTCCCTCGCCAGCATCTCTGTGTGACCCTAAA
keta         ....................................
tshawytscha  ....................................
kisutch      ..............G.....................
clarki       ..............G.....................
salar-NS     ..............G.....................
salar-IRE    ..............G.....................
fontinalis   ..............G.....................
```

b) Microsatellite locus Omy77

```
                                                                    **********************************
mykiss       CGTTCTCTACTGAGTCATGGGGTCAGAGAGAGAGAACAGGGGAGAGAG--AG----------AGAG---AGA
keta                        .......TG.....................--..----------....---...
tshawytscha                 .......TG.....................--.ACAGGGGAGAGAGA.C..GGG...
kisutch                     .......TG.............TA.......AT..----------....---..-
clarki                      .......TG.....................--..----------....---...
salar-NS                    .......TG.....................--..----------....---...
salar-IRE                   .......AG.............--.......--..----------.C..---...

            ******************************************
mykiss       GAGAG--------AGAGAGAGAGAGAGAGAGAGAGAGAGATGC----AGTGAAGCCTTAAAGACCAAGGGTGA
keta         .....--------.....................----------...----.........................
tshawytscha  ....ACAGGGAAG.........................T.....----.........................
kisutch      ---.ACACG----........G..ACAT........--A.C.....AGTG.A..........G.............
clarki       .....--------.....................-----...----.........................
salar-NS     .....--------.....................--------...----.........................
salar-IRE    .G...--------...........AG......----------...----...........GG.............

mykiss       GAGTCTAAATCTTTCATCCTTCTCCCGGATCAGAAAATTCTTGG
keta         ....................
tshawytscha  ....................
kisutch      ..........A.........
clarki       ..........A.........
salar-NS     ..........A.........
salar-IRE    ..........A.........
```

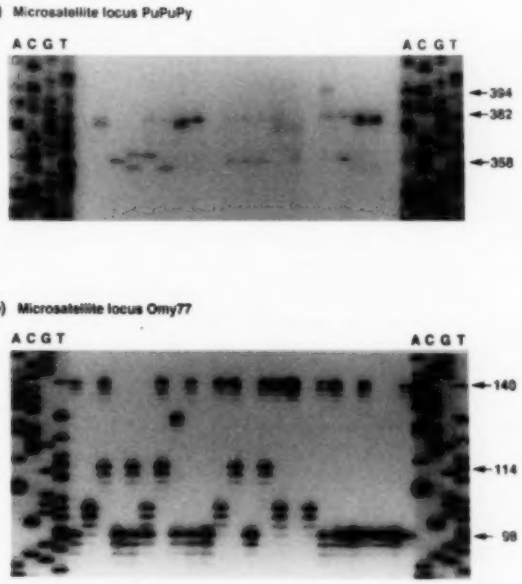
sequencing-type gels containing 8% denaturing polyacrylamide. Gels were fixed in 10% methanol, 10% acetic acid for 20 min, dried under vacuum at 80°C for 45 min, and visualized by autoradiography (Kodak XAR film) for 18 h at –80°C.

Cloning and sequencing of PCR products

In PCR reactions used for cloning, 10 ng of genomic DNA was amplified in a 50-μL reaction under conditions similar to those described above except that the primers (designed with *Eco*RI linkers) were not labelled with radioisotope. Aliquots of the PCR products were size fractionated in 11% polyacrylamide and visualized by ethidium bromide staining.

A 4-μL aliquot of each PCR product was digested with the restriction endonuclease *Eco*RI and ligated into the *Eco*RI site of M13mp18/BAP (Pharmacia). Competent *Escherichia coli* TG1 was transformed with the ligation products and plated on 2xYT medium, containing X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) and IPTG (isopropyl-β-D-thiogalactopyrano-side)

Fig. 3. PCR-amplified microsatellite alleles from several individuals of *O. mykiss* at microsatellite loci PuPuPy and Omy77. Target sites for primers are underlined in Fig. 2. The size of each allele was estimated by reference to co-migrating M13 sequence fragments (A, C, G, and T) run on either side of the trout samples.



(Sambrook et al. 1989). Recombinant clones were sequenced by the dideoxy chain-terminating method (Sanger et al. 1977) using T7 DNA polymerase (Pharmacia). Because the repetitive PuPuPy sequence is very long, the full sequence of the repetitive array could not be readily obtained for most species; however, the full sequences of the flanking regions were determined by employing forward and reverse primers in separate sequencing reactions with double-stranded template (M13 replicative form). Microsatellite PCR products were independently amplified, cloned, and sequenced at least twice from one individual of each species. The sequences flanking the repetitive array were aligned using the program Multalin (Corpet 1989).

Results

General characterization of microsatellites

Seventy-two sequenced clones from *O. mykiss* contained 76 microsatellite sequences that were bracketed by nonrepetitive DNA (Fig. 1). Seventy clones contained GT as the main dinucleotide repeat unit, while 6 contained GA. The majority of sequences were perfect repetitive arrays (61%). Twenty-two percent of the sequences were imperfect, while 17% were compound repeats (12% compound perfect and 5% compound imperfect). The majority of the sequences contained less than 30 repeat units in the longest continuous run (Fig. 1). In total, 24 (32%) of the sequences had more than 30 repeat units. In several instances, the total length of the repetitive array in an imperfect or compound repeat was much longer than the longest continuous run (data not shown), so that the number of large microsatellites was even greater than depicted in Fig. 1. (For example, if a microsatellite contained a sequence such as $(GT)_{30}A(GT)_{25}$, it was categorized according to the arbitrary definitions of Weber (1990) as an imperfect sequence with 30 continuous repeats, although the total length of the marker (with 55 repeats) was substantially longer.)

Table 1. Number of interspecific differences in sequences flanking microsatellites.

	myk	ket	tsh	kis	cla	NS	IRE	fon
myk	-	2	2	8	3	3	6	-
ket	3	-	0	6	1	1	5	-
tsh	3	1	-	6	1	1	5	-
kis	3	2	2	-	5	5	6	-
cla	2	3	3	1	-	0	4	-
NS	5	3	4	2	3	-	4	-
IRE	5	3	4	2	3	0	-	-
fon	6	5	5	3	4	3	3	-

Note: The numbers above the diagonal represent comparisons of 76 bp of sequence flanking Omy77; the numbers below the diagonal represent comparisons of 152 bp flanking PuPuPy. Insertions and deletions were scored as single events. Only the sequences flanking the microsatellites were considered in the comparison because slippage events in the simple sequence array (marked by asterisks in Fig. 2) are expected to create extensive variability within species. myk, *Oncorhynchus mykiss*; ket, *O. keta*; tsh, *O. tshawytscha*; kis, *O. kisutch*; cla, *O. clarki*; NS, *Salmo salar* from Nova Scotia; IRE, *S. salar* from Ireland; fon, *Salvelinus fontinalis*.

PCR amplification of microsatellites in rainbow trout

Two of the microsatellites were analyzed by PCR, with the goal of developing highly polymorphic genetic markers. An extensive survey of allelic length variation was conducted for *O. mykiss*.

The PuPuPy primers amplified 8 alleles over a size range of 36 base pairs (bp) in 114 *O. mykiss* individuals from a hatchery stock in Nova Scotia (Figs. 3*a*, 4*a*). Observed heterozygosity at the locus was 0.78. The Omy77 microsatellite locus exhibited 9 alleles over a size range of 44 bp (Figs. 3*b*, 4*b*), and an observed heterozygosity of 0.72 in 500 different individuals from the same hatchery stock.

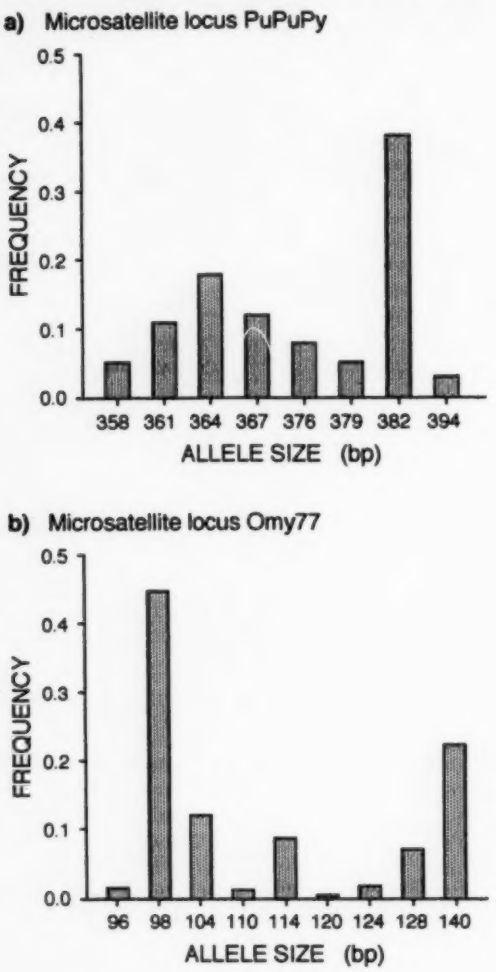
PCR amplification of microsatellites in other salmonids

PuPuPy and Omy77 PCR products were successfully amplified from DNA of 2–6 individuals from each species, except from *Salvelinus fontinalis* DNA, which did not amplify with Omy77 primers (data not shown). Although our sample sizes were small, length polymorphisms at the PuPuPy locus were evident in all species except *Salvelinus fontinalis*. PCR products varied in size by as much as 120 bp within and between species. With the Omy77 locus, polymorphism was evident in all species that displayed an amplification product. PCR products from locus Omy77 varied in size by as much as 90 bp within and between species.

Interspecific comparisons of flanking sequences

Sequencing of the cloned PCR products from each species allowed for the alignment and comparison of the unique sequences flanking the microsatellite arrays (Fig. 2; Table 1). Within the genus *Oncorhynchus*, the PuPuPy flanking

Fig. 4. Allele frequency distributions at microsatellite loci PuPuPy and Omy77 in a hatchery population of *O. mykiss*.



sequence (152 bp) shows a maximum of three differences between species, while the Omy77 flanking sequence (76 bp) shows a maximum of eight differences (Table 1). Differences tend to be higher in comparisons between genera, but this is not always the case, particularly at Omy77 (Table 1).

When compared with *O. mykiss*, the majority of the changes in the unique flanking sequence of PuPuPy in the six other species examined are point mutations, although there is one deletion each in *O. tshawytscha* and *O. keta*. The repetitive array of the PuPuPy microsatellite was not fully sequenced except in *O. mykiss* and *O. tshawytscha*. In *O. tshawytscha* the repetitive sequence formed a perfect tandem array, and the sequence can be summarized as $(PuPuPy)_{114}$. The array was imperfect in *O. mykiss* and can be written as $(PuPuPy)_{11}G_3(PuPuPy)_2A_3GAG(PuPuPy)_{98}$.

When unique flanking sequences at the Omy77 microsatellite are compared with reference to the *O. mykiss* sequence (Fig. 2), *O. keta* and *O. tshawytscha* exhibit one deletion and two point mutations, *O. kisutch* four insertions and five point mutations, *O. clarki* and *Salmo salar* (Nova Scotia) one deletion and three point mutations, and *Salmo salar* (Ireland) one deletion and five point mutations. Sequence data are unavailable for *Salvelinus fontinalis* because of failure of the PCR amplifications with this particular species. In the only comparison within a single species, the two *Salmo salar* sequences differ by four substitutions in the Omy77 flanking sequence, although they are identical in the flanking PuPuPy sequence (Table 1).

Discussion

Characterization of microsatellites in rainbow trout

Microsatellites in rainbow trout display huge variation in the number of repeat units (Fig. 1). The presence of so many sequences with greater than 30 repeat units is unusual when compared with the majority of vertebrate species examined to date (e.g., Stallings et al. 1991; Winterø et al. 1992), but is similar to the distribution seen in other teleosts (Brooker et al. 1994). Brooker et al. (1994) speculated that the long, degenerate microsatellite arrays found in teleosts may result from higher rates of slippage during DNA replication because the organisms are poikilothermic and living in a cold-water environment with severe temperature fluctuations.

Microsatellites as tools for genetic study

The PuPuPy and Omy77 microsatellites reported in this study have been developed as part of an array of markers to be used for the genetic identification of individual fish. These markers have great potential for use in pedigree analysis, population demography, stock assessment, and genomic mapping (Wright 1993; Wright and Bentzen 1994). In a survey of *O. mykiss* individuals from a hatchery stock in Nova Scotia, both microsatellites were very polymorphic with a multimodal distribution of alleles (Fig. 4).

In collaboration with other researchers, the Omy77 locus has also been employed to study genetic diversity in 547 wild *O. mykiss* individuals from 33 streams and 5 hatchery populations in California (Nielsen et al. 1994). Significant differences were observed in allele frequencies between geographic localities, illustrating the power of such markers for population analyses.

Homologous microsatellites in the Salmoninae

Our results suggest that PCR primers designed for microsatellites from one species of salmonid may prove useful for genetic analyses within other species. To demonstrate true homology of the microsatellite markers amplified in different salmonids, we sequenced PCR products from each species. On the basis of a recent revision of the subfamily Salmoninae (cf. Stearley and Smith 1993), the species examined in this study fall into two distinct clades, one that includes the genus *Salvelinus* (the charrs) and one that includes two monophyletic genera, *Salmo* (Atlantic salmons) and *Oncorhynchus* (Pacific trouts and salmons). We found that the unique flanking sequences of both PuPuPy and Omy77 were well conserved among most species in this study, particularly for the PuPuPy locus. The sequences tended to be less divergent within a genus, but were still well conserved between closely related

genera (*Oncorhynchus* and *Salmo*). Both sequences were clearly most divergent when the salmons and trouts were compared with the charrs. Nevertheless, the flanking sequences of PuPuPy and Omy77 appear to be of little use for clarifying the phylogenetic placement of the salmonids, as each locus displayed very few informative sites. Indeed, the variation at Omy77 between the two *Salmo salar* individuals from different populations was actually greater than the variation observed between some species (Table 1).

The limits of the flanking sequence in PuPuPy are easily defined, since the repetitive array seems to end very abruptly. However, inspection of the Omy77 sequences suggests that the sequence adjacent to the repetitive array is influenced by the repeat region, showing evidence of so-called cryptic simplicity (Tautz et al. 1986). This makes it difficult to clearly define where the flanking sequence begins. This is important for interspecific comparisons because variation within any one species is expected to be high in both the repetitive array and the sites of cryptic simplicity, owing to high rates of slippage during DNA replication. The complexity of the flanking sequence also has important implications for the use of microsatellites as genetic tools. If the PCR primers target DNA that is too close to the repetitive array, then the probability of a mismatch (and consequent failure of the primers to anneal) can be high, both within and especially between species. However, if primer sites are too far away from the array, the PCR products become too large for precise resolution of allele sizes by polyacrylamide gel electrophoresis.

The absence of an Omy77 product from *Salvelinus fontinalis* may suggest that the locus arose only after the evolutionary divergence of the charrs from the salmons and trouts. Alternatively, the absence of an Omy77 product may be due simply to sufficient sequence divergence at one or both target sites to prevent the primers from annealing during the PCR. This possibility highlights the concern that the use of heterologous primers may increase the likelihood of undetected null (i.e., nonamplifying) alleles (cf. Callen et al. 1993). Thus, the use of heterologous primers should in general prove most robust when the primers are employed on closely related species or genera, perhaps much less so when employed across higher taxonomic levels.

Polymorphism of the microsatellites in different species

Previous studies using heterologous primers among gadids (Brooker et al. 1994), cetaceans (Schlötterer et al. 1991), and cattle and sheep (Moore et al. 1991) have shown that homologous markers are frequently polymorphic in different species, although markers that appear polymorphic in one species are sometimes monomorphic in another. In this study, polymorphism was evident at both markers in nearly all species. On this basis, we conclude that microsatellite markers isolated from one species of salmonid are, in general, likely to be valuable for studies of other species of salmonid as well. The cross-species utilization of these markers will greatly reduce the development time and costs of genetic research in aquaculture and fisheries.

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Reproduction of walleye in Lake Erie: allocation of energy

Bryan A. Henderson, Jonathan L. Wong, and Stephen J. Nepszy

Abstract: An energetics mechanism is proposed to explain the trade-off between current and future reproduction. Surplus energy can be used for reproductive tissue (current reproduction) or somatic tissue (needed for future reproduction); therefore, we needed to determine first, when does growth of somatic and reproductive tissues occur, and second, how and when is energy allocated to reproduction. Walleye were sampled monthly from the commercial fishery in Lake Erie. Morphological attributes were recorded from these walleye, as well as the lipid composition of a smaller subsample. Energy for reproduction is acquired in the winter, spring, and summer before spawning and stored as visceral lipids. Ovarian lipids are largely derived from visceral fat deposits; thus, females appear to be capital breeders. Females elaborate gonadal tissue only if there is sufficient visceral fat. The circannual endocrine switch to reproduction may occur in midsummer (August) and in October. Females may discontinue ovarian development during the early winter if there is insufficient surplus energy (visceral fat). Year-class variations may be partly explained by the energy condition of the females and the proportion of the stock able to spawn successfully each year.

Résumé : Nous proposons un mécanisme énergétique pour expliquer le compromis entre la reproduction actuelle et future. Le surplus d'énergie peut servir à constituer du tissu reproducteur (reproduction actuelle) ou du tissu somatique (nécessaire pour la reproduction future); nous devions donc déterminer premièrement quand se produit la croissance des tissus somatiques et reproducteurs et, deuxièmement, comment et quand l'énergie est affectée à la reproduction. Nous avons prélevé chaque mois des échantillons dans les captures commerciales de dorés du lac Érié. Nous avons consigné les caractéristiques morphologiques de ces poissons, ainsi que la composition lipidique d'un sous-échantillon plus petit. L'énergie destinée à la reproduction est acquise pendant l'hiver, le printemps et l'été, avant la fraye, et est stockée sous forme de lipides viscéraux. Les lipides ovariens proviennent en bonne partie des dépôts de graisse viscérale; les femelles semblent donc jouer un rôle crucial dans la reproduction. Elles élaborent du tissu gonadique seulement si la quantité de graisse viscérale est suffisante. Le tournant endocrinien circannuel vers la reproduction peut se produire au milieu de l'été (août) et en octobre. Les femelles peuvent interrompre leur développement ovarien pendant le début de l'hiver si l'énergie excédentaire est insuffisante (graisse viscérale). Les variations des classes d'âge peuvent s'expliquer en partie par la condition énergétique des femelles et par la proportion du stock qui est capable de se reproduire avec succès chaque année.
[Traduit par la Rédaction]

Introduction

Walleye (*Stizostedion vitreum*) abundance in Lake Erie decreased markedly in the late 1950s, primarily because of a largely inexplicable and progressive deterioration in recruitment starting in the mid-1940s (Parsons 1967, 1970). The effects of water and habitat contamination, exacerbated by exploitation, were associated with the decline, but the mechanism was not clear (Parsons 1970). Blue walleye (*Stizostedion vitreum glaucum*) did not recover in the eastern portion of Lake Erie, but the yellow walleye (*Stizostedion vitreum vitreum*) did recover in western and central Lake Erie in the 1980s (Hatch et al. 1990), largely as a result of a few strong year-classes in 1977, 1982, 1986, and 1991 (Henderson and Wong 1991). Annual recruitment varied considerably and yields have been dependent upon the appearance of good year-classes every 3–5 years. Recruitment was shown to be affected by wind, water temperatures, and spawning stock (Busch et al. 1975; Shuter and Koonce 1977), but generally was highly unpredictable. Henderson and Nepsy (1994) suggested that year-class strength may also be associated with the energy condition of the mature females. At present the fishery is managed for optimal yield consistent with an assumed natural mortality rate, without any explicit consideration given to the reproductive potential of the stock. In this study our aim was to develop a model of a mechanism that could explain variations in reproductive effort in terms of the costs of reproduction, constrained by the energy needed to mature and continue to spawn annually. Thus, we were trying to provide another explanation for annual variations in recruitment.

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Table 1. Date, location, sample size, and sampling gear for subsample of walleye from commercial walleye catch in Lake Erie (June 1992 – May 1993).

Month	Number	Basin	Gear
January	45	Western	Commercial
February	50	Western	Commercial
March	39	Western	Commercial
April	49	Western	Commercial
May	47	Western	Commercial
June	45	Western	Commercial
July	61	West-central	Commercial
August	44	Central	Commercial
September	43	West-central	Commercial
October	36	Western	Experimental
November	122	Western	Commercial
December	54	Western	Commercial

Life history theory tries to explain why and how an optimal level of reproductive effort is achieved (van Noordwijk and de Jong 1986). The optimal value is constrained mostly by the costs of reproducing; as a consequence there is a trade-off between current reproduction versus survival and future reproduction (Stearns 1992). Life history theory is principally concerned with trade-offs determining optimal fitness on physiological, microevolutionary, or macroevolutionary scales (Stearns 1992). The implication from Henderson and Nepszy (1994) was that some walleye failed to reproduce in the current year because there was a trade-off between current and future reproduction. Residual reproductive value is reduced if elevated mortality is the consequence of spawning. Here we recognize two major constraints on the realization of reproductive value. The first constraint consists of the physiological and anatomical traits that have evolved in response to historical selective pressures; evolutionary trade-offs have resulted in a genetically determined life history tactic that can be considered a constraint in itself. The second constraint is the amount of surplus energy available to realize reproductive value within the context of these previously selected physiological and anatomical traits. The energy demands of spawning and the ability to recover energy deficits could affect both mortality and the ability to spawn in consecutive years. Thus, recruitment may be partly dependent upon the proportion of the spawning stock that can effectively mature each year as a consequence of the energy acquired before spawning, including the recovery of energy from previous spawning (Henderson and Nepszy 1994). In effect we are trying to understand the physiological and energetic mechanism for the microevolutionary trade-off between current and future reproduction, and the plasticity of this tactic to annual variations in availability of energy. Thus, how are variations in recruitment a function of both past evolutionary pressures and present environmental conditions? Our approach was to develop a conceptual model of the circannual acquisition and allocation of energy for growth and reproduction as has been done for a few other species (Craig 1977; Diana and MacKay 1979; Hayes and Taylor 1994). We have focussed on the role of lipid dynamics as the mechanism regulating reproductive development (Frisch 1988) and the anatomical attributes that reflect circannual patterns of lipid composition and allocation, such as somatic lipids, liver, visceral fat stores, and reproductive tissue.

The first hypothesis is that (*i*) reproduction by females is constrained by the acquisition of energy prior to the elaboration of gonadal tissue and (*ii*) energy is diverted to reproduction from stored energy (capital breeders), rather than being derived from feeding (income breeders) during ovarian development (cf. Sibly and Calow 1986). In particular, we hypothesized that the energy used to elaborate ovarian tissue was derived from visceral fat stores. When is this surplus energy acquired and when is it transferred to reproductive tissues? We have investigated these hypotheses and questions by describing the allocation of lipids throughout the year as the walleye grew and sexually matured. Thus, we have been able to document when energy was acquired and how energy was allocated to reproduction.

Methods

General comments

An important assumption of this study was that we were able to obtain an unbiased sample of the population for any particular age-class; biased sampling was reduced in part by our length-stratified subsampling of the catch. All of our subsamples were obtained by commercial gill nets with a stretched mesh of about 102 mm, with equal numbers of males and females selected from 8 size-classes in 5-cm intervals from 30 to 65 cm fork length. Thus, the size composition of our subsample did not change throughout the year. Futhermore, we showed that the proportion of lipids in walleye is independent of body size, so that changes in circannual variations in lipid composition do not appear to be influenced by the selectivity of gill nets. We also assume that lipid and energy dynamics are, for the most part, comparable during the normal course of events (Love 1960, 1970; Damberg 1964; Reshetnikov et al. 1970; Newsome and Leduc 1975; Campbell and Love 1978) except during starvation when protein catabolism can become increasingly important as a source of energy (Love 1960; Cunningham and Shuter 1986).

Sampling design and attributes

Morphological and anatomical attributes and, most importantly, lipid composition of walleye were assessed monthly from June 1992 to May 1993. From these data we derived most of the important life history traits of growth pattern, size and age at maturity, and age- and size-specific reproductive investments; this permitted us to comment on the trade-offs between current reproduction and both survival and future reproduction. Walleye (100 of each sex) were sampled from Lake Erie (42°15′N, 81°15′W) commercial gill-net catches at the beginning of each month; 3 or 4 of each sex were then subsampled from each 50-mm fork-length class from 300 to 700 mm for detailed measurements of organ weight, visceral fat, and lipid composition (Table 1). In October, the walleye sample was derived from catches in experimental gill nets (Henderson and Wong 1991). The distribution of samples corresponded to the annual geographical distribution of walleye; for the most part, walleye migrate from the western basin after spawning to the central basin for the summer, and then move back to the

Fig. 1. Monthly growth in round weight (g) from age 2 (24 months) to age 10 (120 months) for females (A) and males (B) for combined year-classes.

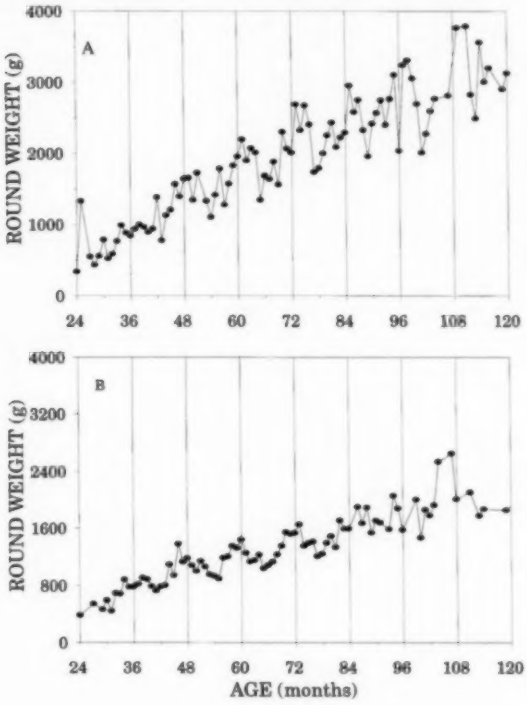
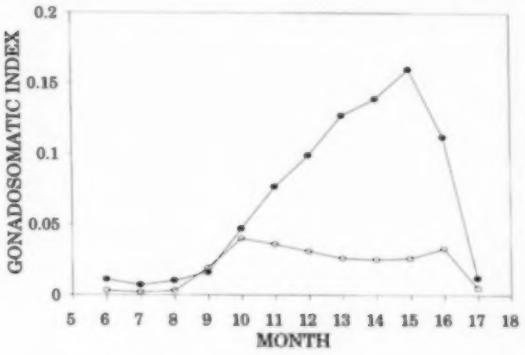


Fig. 2. Gonadosomatic index (GSI) for females (●) and males (○) from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.



western basin during the early autumn (B.A. Henderson and J.L. Wong, unpublished data).

From all of the sampled walleye, attributes were measured to estimate life history traits: fork length (mm), round weight (g), age from scale impressions on acetate slides, sex, maturity, gonadal development, gonad weight (g), and liver weight (g). From the subsample (Table 1), fecundity and lipid composition were assessed. Fecundity was estimated by counting the number of eggs in 1-g subsamples removed from the ovary.

Lipid extraction

The method of lipid extraction (methanol–choroform) was modified from the methods of Bligh and Dyer (1959). The entire carcass, without stomach contents, was homogenized, except when the gonads were analyzed separately. From a wet subsample (100 g) of the homogenate, the dry weight proportion was estimated by drying the sample at 105°C for 24 h. Approximately 2 g of dried homogenate (total carcass, somatic tissue, or gonadal tissue) was transferred to a 125-mL Erlenmeyer flask, to which was added 14 mL of distilled water, 40 mL of methanol, and 20 mL of chloroform. This mixture was homogenized for 3 min in a tissue homogenizer before it was vacuum filtered through a Buchner funnel with Whatman No. 1 filter paper; a small amount of Whatman filter-aid was added to facilitate the removal of the largest particles. The washes from the tissue homogenizer (20 mL of chloroform) and the original Erlenmeyer flask (homogenate, water, chloroform, and methanol) were also filtered. The clear filtrate was then transferred to a 100-mL graduated cylinder containing 25 mL of distilled water; this mixture was allowed to stand for 5 h before the volume of the lower chloroform–lipid layer was recorded both before (volume 1) and after (volume 2) the water–methanol layer was removed by suction. The lipid fraction was then transferred to previously weighed 50-mL beakers and air dried in a fume-hood for 12 h before being dried for 2 h at about 100°C in a drying oven. Then the lipids and beaker were weighed. The proportion of fat in the dried sample was estimated as follows:

$$\mathrm{PDL} = \frac{\mathrm{DFW}}{\mathrm{DSW}} \cdot \frac{\mathrm{V1}}{\mathrm{V2}}$$

where PDL is the proportion dry lipids, DFW is the dry fat weight, DSW is the dry sample weight, V1 is volume 1, and V2 is volume 2.

Results

Seasonal changes in gonad, liver, and round weight

The weight of males and females at age diverged near the end of the 4th year of life (46 months); females (Fig. 1A) approached an asymptotic size by about the 10th year, and males by the 8th year (Fig. 1B).

Gonadal development of mature walleye, expressed as the weight of the gonad divided by the round weight, began between August and September for both sexes (Fig. 2). Ovarian size increased until March (gonadosomatic index (GSI) = 0.16,) and the subsequent decline in size was associated with spawning. Variability of GSI tended to increase during development; the bimodal size distribution in April (Fig. 3) was caused by early spawning. In April, fecundity was linearly related to both ovary weight (eggs = –848.138 + 411.7 ovary weight (g), df = 21, r^2 = 0.63) and round weight (eggs = –6 4546.2 + 96.9 round weight (g), df = 19, r^2 = 0.70). Testicles reached a peak size by October (GSI = 0.040) and then decreased in size to April (GSI = 0.033), before declining abruptly in size at spawning.

The hepatosomatic index (HSI), calculated as the wet weight of the liver divided by round body weight, began to increase between July and August for both sexes (Fig. 4). Female HSI peaked (0.032) in February and then decreased

Fig. 3. Distribution of individual gonadosomatic indices (GSI) of females from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.

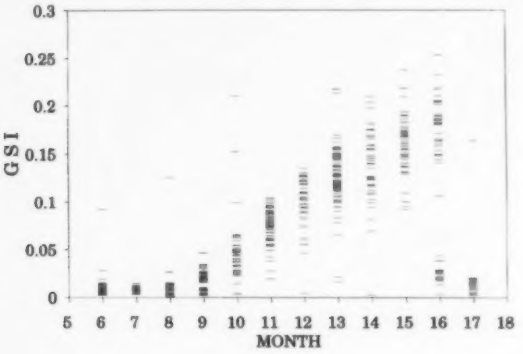


Fig. 4. Hepatosomatic index (HSI) of mature females (●) and males (○) from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.

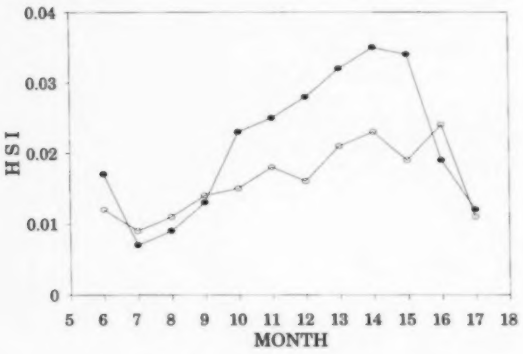
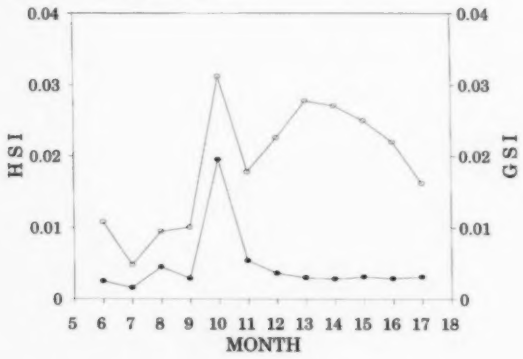
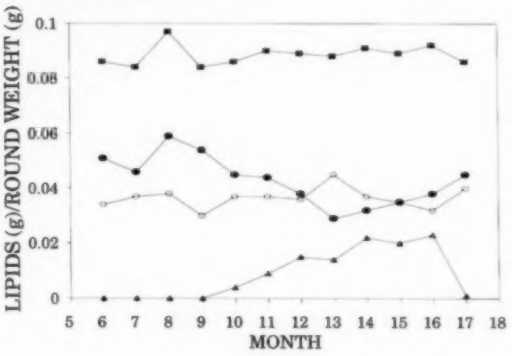


Fig. 5. Hepatosomatic (HSI, ○) and gonadosomatic (GSI, ●) indices of immature females from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.



quickly between March and May; male HSI increased more slowly to a peak (0.024) by April then decreased to a value (0.011) comparable to that of females (0.012) by May; the lowest HSI was observed in July. The liver was probably increasingly active in the elaboration of lipoproteins and other constituents deposited in the gonads. Among the females, the largest monthly increment in liver size (September–October) was coincident with the initial increase in the size of the ovary (Fig. 4).

Fig. 6. Lipid density (wet lipid/round weight (g)) of total carcass (■), somatic tissue (○), visceral fat (●), and ovary (▲) for mature females from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.



The assessment of maturity of females was subjective; relative ovary mass and egg size were small for immature females. It is possible, but unlikely, that these immature females could have matured much later. The monthly variation in GSI and HSI for these immature females was similar to that of mature females up to October, but then the patterns diverged (Fig. 5). The GSI of immature females did not increase to the same level as that of mature females by October. In contrast, the GSI of mature females continued to increase, whereas the GSI of immature females dropped to levels seen during the summer. The HSI of immature fish also dropped, rising again to higher levels, but not rising to the levels normally recorded for mature females.

Seasonal allocation of lipids in somatic, visceral, and gonadal tissue

Female total lipids (wet lipids (g)/round weight (g)) remained at about 0.09 throughout the year, except for a transitory peak in August (Fig. 6). Similarly, somatic lipids were relatively constant except for a decline from January (0.048) to April (0.033). Lipids stored as visceral fat peaked briefly in August, then decreased as the total lipids in the ovary increased. Subsequently, the visceral fat deposits increased in the late winter and early spring (January–May). The ovarian lipid level increased between September and October, reaching an asymptote by February (Fig. 6). Thus, it appeared that the largest portion of the lipids in the ovaries was probably derived from the visceral fat. This interpretation is largely based on the observation that ovarian lipid levels increased as visceral fat levels declined. However, the last increase in ovarian lipid level (January–February) was not at the expense of visceral fat levels. Energy derived from feeding in the early winter may have explained the increase in both visceral fat and ovarian lipid levels from January to February.

The monthly variation in the lipid density of immature

Fig. 7. Lipid density (wet lipid/round weight (g)) of total carcass (■), somatic tissue (○), and visceral fat (●) for immature females from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.

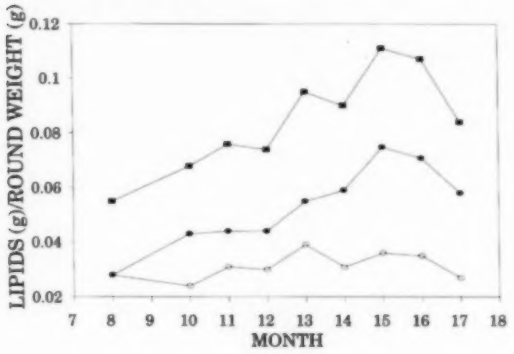
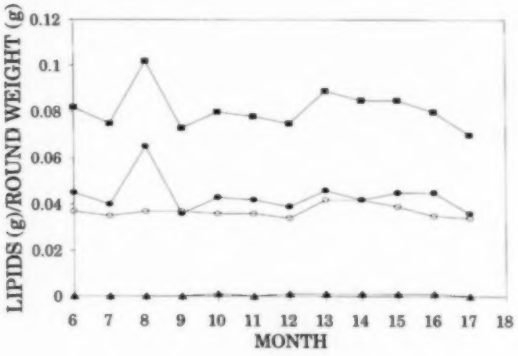


Fig. 8. Lipid density (wet lipid/round weight (g)) of total carcass (■), somatic tissue (○), visceral fat (●), and testes (▲) for mature males from June 1992 (month 6) to May 1993 (month 17) in Lake Erie.



females (Fig. 7) was strikingly different from that of mature females. In the early autumn (October), the lipid density in the visceral tissue did not differ much from that of mature females, but the lipid density in the somatic tissues was much lower (immature, 0.024; mature, 0.038). Somatic lipid density increased slightly over the winter, but the visceral lipid density in immature females increased markedly to higher levels than that in mature females by March (immature, 0.075; mature, 0.035).

Male total lipids (wet lipids (g)/round weight (g)) were marginally lower and more variable than female lipid levels (Fig. 8). Males also showed a transitory peak in August (0.102). Total lipids declined (0.073) by September and then recovered between December and January (0.89), followed by a progressive decline in lipid levels until March (0.070). Most of the last decline in total lipids was explained by a loss of somatic lipids, except for a drop in visceral fats from April to May (Fig. 8). Thus, the males started spawning with more available energy (wet visceral fat + wet somatic lipids = 0.080; lipids (g)/round weight (g)) than females (0.070) and finished

Fig. 9. Regression of total carcass lipid wet weight (g) on total visceral fat (g) for both males and females from Lake Erie.

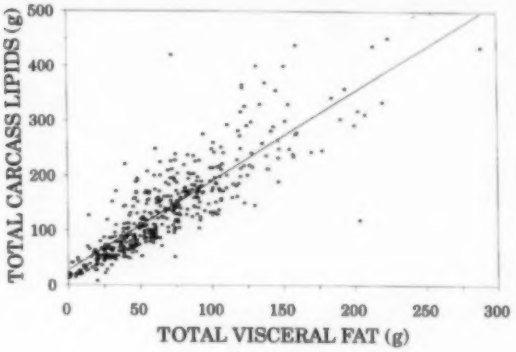
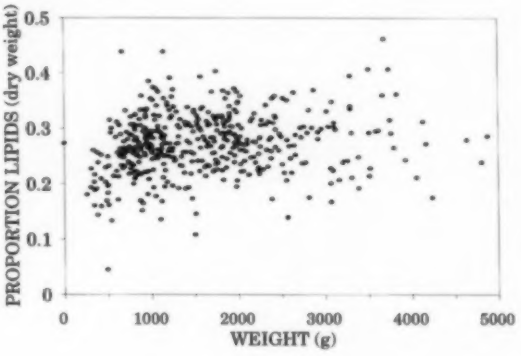


Fig. 10. Proportion of lipids, by dry weight, in relation to wet round weight for pooled sample of male and female walleye from Lake Erie.



spawning with less total energy (males, 0.070; females, 0.085). Females may have resorbed some energy from ovarian tissue after spawning.

More than 60% of total lipids for both males and females was stored as visceral fat (Fig. 9; total lipids (g) = 1.631 visceral fat (g) + 27.03, df = 451, $r^2 = 0.64$). More of the variation in total lipids (TL) was explained by visceral fat levels (VF) for males (TL = 1.467 VF + 22.83, df = 185, $r^2 = 0.82$) than females (TL = 1.583 VF + 39.81, df = 264, $r^2 = 0.55$), because there was more variation in visceral fat level amongst females during the year. For walleye greater than 1100 g in weight, the proportion of lipids was not correlated with the size of the walleye (Fig. 10).

Discussion

The Panglossian paradigm is that life history traits are optimally adapted to the environment, consistent with the best possible lifetime reproductive value (Gould and Lewontin 1979). This paradigm fails to recognize that selection does not account for all morphological and physiological characteristics that may arise from genetic drift and allometry. Despite these drawbacks, life history theory still provides a

framework for a coordinated interpretation of many of the attributes of populations (mortality, recruitment) and individuals (growth, maturation, fecundity). In this study we have used the conceptual framework of life history theory (Stearns 1992; Roff 1992) to develop hypotheses about the physiological and environmental constraints affecting reproductive values of walleye. Ultimately, we believe that an understanding of the evolutionary and environmental constraints on reproductive value will help with the management of walleye populations in diverse environments. Here we are concerned only with walleye in Lake Erie (a productive and diverse fish community), but we anticipate that the results will provide some insight into the reaction norms of walleye found in less productive and diverse fish communities in the cooler, northern parts of Ontario.

First, are walleye capital or income spawners? More specifically, is reproduction by female walleye constrained by their ability to acquire sufficient energy for the elaboration of the ovary, before gonadal development begins in the early autumn? Second, if walleye are capital breeders, is there a time when the endocrine system either switches to normal gonadal development or alternatively switches to gonadal resorption if there is insufficient energy for vitellogenesis? Capital and income spawners may represent the ends of a continuum; species may compensate for inadequate capital (lipid deposits) with income derived by feeding.

Energy density of visceral fat varied most amongst mature females during the year. Visceral fat may constitute the bulk of surplus energy that is allocated for reproductive effort (Leon 1976; Schaffer 1974). Female walleye appear to be more capital than income spawners. Visceral fat levels fell from August to January and thereafter increased. The fall in visceral fat levels was associated with most of the increase in ovarian size and ovarian energy density. In contrast, the male visceral fat level was generally lower and relatively constant for most of the year. Ovarian development was initiated after a surplus energy store was replenished. Since visceral fat levels fell as ovarian lipid level increased, visceral fat deposits were probably the main source of lipids deposited in the ovary. Thus, surplus energy was acquired from January to August, and then allocated to ovarian development from August to January.

Is there a circannual endocrine switch from reproductive dormancy to active gonadal development, which is triggered by the energetic status of the female? The test of this hypothesis was weakened by our methodology because we were not monitoring the energy condition and gonadal development of individual females. Hence, we could not determine if females with low energy reserves stopped elaborating gonadal tissue at some stage of development after the summer accrual of stored energy; rather, we were sampling the energetics and reproductive status of the population. However, only females with sufficient surplus energy continued with gonadal maturation as suggested by Henderson and Nepszy (1994). In this study, immature females had lower visceral fat stores in the early summer when gonadal development began. However, there was a smaller, transient increase in the size of the ovaries of these immature females until October, after which the ovarian weight declined to a dormant size. The liver increased to similar sizes in both immature and mature females until October (Figs. 4, 5), then the liver size of the immature females declined. Assuming that the liver is the conduit for the transfer of lipids from the visceral deposits to the ovary (White et al. 1968), this variation in liver size was probably related to the allocation of lipids to the ovary. Although liver size was correlated with gonadal size, it was not necessarily correlated with lipid mobilization rates. During March and April when HSI was maximal, there was no evidence of lipid mobilization. Perhaps the continued increase in liver size was related to other high-energy costs of reproduction. Thus, our data point to two separate times when an endocrine switch could operate: first, after the surplus energy store (visceral fat) is replenished by August, and second, in October when even immature females exhibited a transient development of ovarian tissue.

Visceral lipid deposits seemed to be the most important source of lipids destined for the ovaries. We propose that there is some physiological mechanism (Frisch 1988) for assessing the availability of lipid energy, and if the visceral lipid deposit was below some level, then gonadal development ceased. In fact, the level of visceral fat found in females during the early autumn may be a useful indicator of condition and spawning potential. Possibly, this mechanism could operate at a later stage of ovarian development if the visceral lipid deposits were depleted by the early winter. We were monitoring the development of the population and not individuals, but some of the lower values of GSI observed during the early winter could be explained by the individual reduction of ovarian size as energy was absorbed for other metabolic needs. The pause in the elaboration of ovarian lipids seen from December to January may be a reflection of a period when the reduction in visceral lipids resulted in a temporary cessation of ovarian growth; ovarian lipid levels increased again only as the visceral lipid deposit increased. The increase in visceral fat stores may vary depending on the availability of prey during the winter; both water temperatures and prey density and distribution would affect prey availability. Thus, although the walleye was primarily a capital spawner, income from feeding could provide energy for gonadal development at later stages.

Of what value is the description of the life history strategy of female walleye to a fisheries manager? The conservation of minimum viable stocks of walleye when faced with exploitation or habitat alteration requires information about the risks of extinction of small populations and the characteristics of the spawning populations. For the most part, effective spawning populations may be smaller and more variable than expected, if female maturation is delayed or intermittent, depending on the energetics status of the females. Thus, the apparent residual reproductive value may be overestimated because we underestimate the cost of spawning. This may partly explain the poor relationship between spawning stock size and recruitment (Shuter and Koonce 1977). As suggested by Henderson and Nepszy (1994), recruitment variations may be associated with the energetic condition of the stock. If a harvest was taken from a spawning population that was a fraction of the estimated one, then the acceptable fishing mortality could be too high. Furthermore, the reproductive value of the mature females would be much reduced if fishing mortality were to be maintained at a level based on an incorrect assumption that the population spawns annually after first maturation. The evolved life history strategy depends on the assumption that longevity (as a function of mortality) is increased as spawning frequency declines; senescence may constrain the effectiveness of this strategy by setting an upper limit to the potential number of

spawning episodes. The risks of extinction are then greater for stocks with small spawning populations with lower probabilities of multiple spawning episodes.

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Biofilm and substrate preference in the dreissenid larvae of Lake Erie

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Abstract: Larval recruitment in the bivalves *Dreissena polymorpha* and *Dreissena bugensis* was measured on acrylic plastic plates with live mussels, mussel shells, or stones with primary biofilm and with the primary biofilm removed by scrubbing. Experiments were carried out at Nanticoke Thermal Generating Station on Lake Erie for nine 24-h periods between 21 July and 5 August 1993. Recruitment density ranged from a high of 29 300 ± 34 800 mussels·m^{-2}·day^{-1} (mean ± SD, n = 10) for plates with shells and biofilm to a low of 4640 ± 540 mussels·m^{-2}·day^{-1} (n = 10) for the stone treatment that had the biofilm removed by scrubbing. Recruitment was not significantly different between treatments with dreissenid shells and live adult dreissenids though both of these treatments had more settling than the plates with mussel-sized stones. Our results show that dreissenid shell material is a particularly attractive substrate for dreissenid recruitment in Lake Erie and that biofilm removal reduces mussel recruitment by 10–20%.

Résumé : Nous avons mesuré le recrutement des larves des bivalves *Dreissena polymorpha* et *Dreissena bugensis* sur des plaques d'acrylique portant des moules vivantes, sur des coquilles de moules ou sur des pierres, en présence du film biologique primaire ou après enlèvement de ce film biologique par raclage. Les expériences ont été menées à la centrale thermique de Nanticoke, sur le lac Érié, pendant neuf périodes de 24 h, entre le 21 juillet et le 5 août 1993. La densité de recrutement allait d'un sommet de 29 300 ± 34 800 moules·m^{-2}·jour^{-1} (moyenne ± ET, n = 10), pour les plaques portant les coquilles et le film biologique, à un plancher de 4640 ± 540 moules·m^{-2}·jour^{-1} (n = 10) dans le cas des pierres dont on avait enlevé le film biologique par raclage. Le recrutement n'était pas nettement différent entre les expériences avec les coquilles de dreissinidés et les adultes vivants, mais dans les deux cas le naissain était plus nombreux que sur les pierres de même taille. Nos résultats montrent que les coquilles de dreissinidés constituent un substrat particulièrement attirant pour le recrutement de ces mollusques dans le lac Érié, et que l'élimination du film biologique réduit de 10–20% le recrutement des moules.
[Traduit par la Rédaction]

Introduction

The zebra mussel (*Dreissena polymorpha*) and its close relative the quagga mussel (*Dreissena bugensis*) have become serious economic biofoulers since their accidental introduction into the Great Lakes (Hebert et al. 1991). The recruitment and aggregation of these exotic dreissenids has led to the occlusion of domestic and industrial pipelines, electrocorrosion of steel pipes (Mackie et al. 1989), water outages (LePage 1993), and fouling of trash bars, condensers, and heat exchangers (Kovalak et al. 1993). Estimates of damage caused by dreissenid infestations are in excess of $5 billion before the year 2000 for the Great Lakes region (Armor and Wiancko 1993).

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The Lake Erie dreissenids are diecious with gametes released directly into the water and fertilized externally. After a brief trocophore stage, free-swimming veliger larvae are produced (Walz 1975). At the time of settlement and metamorphosis (i.e., recruitment) the larval mussels secrete byssal threads that allow them to attach to most hard substrates. Once attached, mussels may detach from their byssal threads and move to another substrate (Martel 1993). However, the significance of this translocation behaviour to the overall population is difficult to assess. Inhibition of recruitment offers a promising means of controlling dreissenid infestation, since recruitment is the first step in biofouling. To efficiently control exotic mussel recruitment, colonization and the mechanisms of substrate selection must be understood.

Recruitment of many sessile marine invertebrates on or near conspecifics is critical as it can increase reproductive success, filter feeding efficiency, and competitive ability, and decrease juvenile and adult mortality (Knight-Jones and Stevenson 1950; Knight-Jones and Crisp 1953; Bayne 1969; Hidu 1969; Meadows and Campbell 1972; Tamburri et al. 1992; McGrath et al. 1988; reviewed by Williams 1964 and Rodriguez et al. 1993). There are few similar data for freshwater mussels but aggregations of zebra and quagga mussels are common, consisting of clumps of individual mussels attached to each other by byssal threads (Mackie et al. 1989) with populations in excess of 750 000 individuals·m^{-2} (Kovalak et al. 1993).

Studies have shown that free-swimming larvae of many marine invertebrates make active, consistent habitat choices and that recruitment is seldom random (reviewed by Bayne 1969; Meadows and Campbell 1972). For example, the recruitment of planktonic larvae can be affected by light, gravity, surface texture (Crisp 1974), current velocity, initial surface chemistry, and chemical cues from bacteria and microflora (Meadows and Campbell 1972; reviewed by Roberts et al. 1991).

Since 1935, when Zobell and Allen noted that surfaces were more attractive to settling larvae when microbial films were present (Bonar et al. 1990), there has been considerable research on the role of surface biofilms (reviewed by Tamburri et al. 1992) in inducing settlement. Studies have shown that in the marine environment bacteria and other microorganisms quickly colonize submerged surfaces (Maki et al. 1988, 1989, 1990). The surface biofilm initially consists of a glycoproteinacious film. Soon after immersion a surface is colonized by bacteria, diatoms, and protozoa (the primary film). It has been shown that certain marine bacteria increase recruitment of a number of marine organisms (reviewed by Williams 1964; Meadows and Campbell 1972; Maki et al. 1988, 1990; Harrold et al. 1991; Johnson et al. 1991).

While this phenomenon is well known in marine research, there is no documented research on the effect of biofilms on the recruitment of zebra or quagga mussels. The nature of the stimulus that leads to the recruitment and aggregation of dreissenids is also unclear. The objectives of this study were to determine if surface biofilms affect dreissenid recruitment and to investigate if adult conspecifics or their shells influence the recruitment pattern of veligers.

Methods

Study site

All experiments were carried out in the forebay of the Nanticoke Thermal Generating Station (T.G.S.) at Nanticoke, Ontario, on Lake Erie (42°48′N, 80°03′W). Water in the forebay is pumped from Lake Erie through two 600-m tunnels from a depth of 13 m. The tunnels and the forebay are extensively colonized with dreissenids, and mussel fouling of condensers and pump intakes is a major problem for the generating station. Dreissenids collected from the walls of the forebay are 95–100% *D. bugensis*.

Experimental methods

Six experimental treatments were chosen to evaluate the effect of biofilms and adult dreissenids on veliger recruitment. Each treatment consisted of a substrate, either live mussels, dreissenid mussel shell, or stones, held on 5 × 10 cm acrylic plastic (Plexiglas™, methyl methacrylate) plates by nylon 0.5-cm mesh fabric bags. For each type of substrate there was a biofilm and a biofilm-removed treatment. The substrate for the first treatment was live adult dreissenids with biofilm removed by scrubbing (see next section); the second treatment was identical except that the biofilm was not removed from the adults and the acrylic plate. These live-mussel treatments were used to investigate whether a substance from live adult dreissenids induced recruitment of veligers. The substrate in treatment 3 consisted of dreissenid shells on plates with biofilm removed; the fourth treatment was identical to the third except that the biofilm was left intact on the shells and plate. Treatments 3 and 4 were used to determine if the dreissenid shell alone could change recruitment rates. Treatment 5 consisted of stones on an acrylic plate with biofilm; in treatment 6 the biofilm was removed from the stones and the plate. The stones in treatments 5 and 6 were intended to simulate the hydrodynamic qualities of adult mussels and act as a control group.

The adult dreissenids used in treatments 1 and 2 were collected attached to rocks in Nanticoke Harbour on 8 July 1993, before any apparent recruitment in eastern Lake Erie. These 2- to 3-cm zebra and quagga mussels were kept in the laboratory in recirculating 50-L aquaria where they were fed every 2–3 days with *Chlorella pyrenoidosa* to a concentration of 30 000 cells·mL^{-1}. The water in the aquaria was replaced with fresh Lake Erie water every 3–4 days. Mussels used for the experiments were cut from their colonies with a scalpel and were used in the experiments only after they opened and extended their siphons. Four adult mussels where confined on each surface of the acrylic plastic plate in treatments 1 and 2.

Preparation of dreissenid shells and stones

The mussel shells used in treatments 3 and 4 were collected from trash racks at Nanticoke T.G.S. that had been removed the previous year. The shells were cleaned by sonication for 3–4 h then scrubbed with a brush and Sparkleen™ and then rinsed five times in deionized water before use. In treatments 5 and 6, eight mussel valves were confined, inside surface down, on the surface of the acrylic plates.

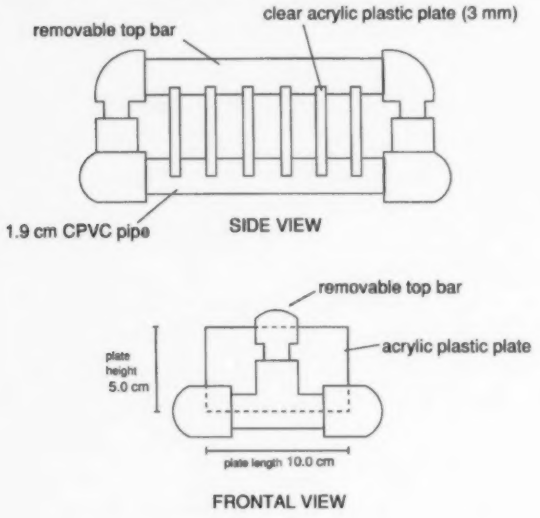
Stones used in treatments 5 and 6 were between 1 and 3 cm in diameter and approximately the size and shape (oblong and compressed) of the adult dreissenids used in treatments 1 and 2. Stones were cleaned in the same manner as the dreissenid shells. Four stones were confined on each face of the acrylic plates.

Preparation of surface biofilms

Before experiments began, a biofilm was allowed to develop on the various substrates in 30-L aquaria for 4–6 days under the continuous light of a 40-W Gro and Sho™ fluorescent light placed 15 cm above the surface of the tank. Tanks were filled with water collected at Nanticoke T.G.S. Biofilm was grown on the substrate and acrylic plates after they had been contained in the nylon mesh bags.

Biofilm and other surface material in treatments 2, 4, and 5 were removed by the method of Tamburri et al. (1992) with a few modifications. In our experiments biofilm was removed from substrate surfaces and plates by scrubbing with a soft, nylon-bristled brush and Sparkleen (Fisher Scientific, Pittsburgh, Pa.), a moderately alkaline, free-rinsing detergent blend designed for manual washing. We elected to use a detergent rather than the sodium hypochlorite used by Tamburri et al. (1992) for two reasons. Hypochlorite ion is extremely toxic to zebra mussels (Martin et al. 1992) and we felt that its use could lead to unacceptable levels of mortality among the live mussels. Also, hypochlorite ion is extremely reactive and we felt that it may oxidize shell biochemicals critical to settlement. All materials that we had cleaned with detergent were rinsed five times with distilled water to remove any traces of soap or surfactant. It is likely that no residues were left on the cleaned surfaces because Sparkleen leaves no inhibitory or toxic

Fig. 1. Diagram of the experimental apparatus used for containing acrylic plastic plates. All parts of the apparatus are made of chlorinated polyvinyl chloride plastic (CPVC).



residues when assayed using the glassware inhibitory residue test (American Public Health Association, American Water Works Association, and Water Environment Federation 1992) by Scientific Associates Inc., St. Louis, Mo.

Once the shells and stones had been cleaned and rinsed they were stored for 18–24 h in deionized water before being installed in the racks. Live, scrubbed mussels were held for 18–24 h in a mixture of 50% unchlorinated, Guelph well water filtered through Whatman GF/F filters and 50% deionized water. No mussel mortality resulted from the cleaning process.

Groups of six plates (one of each treatment) were randomly placed in slotted racks constructed entirely of chlorinated polyvinyl chloride plastic (CPVC, see Fig. 1). Plates in racks were 3 cm apart and three racks (18 plates) were used on each experimental date.

For each experiment, racks were randomly suspended 1–1.5 m out from the corrugated metal wall of the forebay and 2 m below the surface of the water. Ten experiments were carried out between 20 July and 10 August 1993. Racks were placed in the water between 11:00 and 14:00 and removed 23.5–24.5 h later and air dried.

Newly recruited dreissenids were counted under 400× magnification using a Wild–Heerbrug stereomicroscope. The newly recruited dreissenids showed up as pearlescent spheres against the darker background of the plates and substrates. We could not distinguish between the newly recruited mussels of the two dreissenid species in Lake Erie though this may now be possible (Martel 1995). During periods of intense settling, clumps of 10–15 newly recruited mussels had to be teased apart and counted. Only mussels found on the substrates and the large flat surfaces of the plates (not on the edges of the plates or on the mesh bags) were counted. An areal recruitment value in mussels per square metre for each treatment was arrived at by adding together recruitment on the substrate and the 100-cm^2 acrylic plastic plate and multiplying by 100.

The shell length of the recruits was measured on 20 July, 29 July, and 9 August 1993. Measurements were made at 160× magnification using a Hi-pad digitizing tablet interfaced to an IBM personal computer (Roff and Hopcroft 1986). The length was measured as the longest distance between the umbone and the ventral margin. Mussels larger than 500 μm were considered to be translocators (mussels that had metamorphosed and, perhaps, previously settled) and were excluded from the counts. No more than two translocators were ever detected per treatment.

To assess recruitment patterns over a longer term, additional experiments, using the same methods as outlined above, were carried out over 7 or 10 days rather than 24 h. These experiments commenced on 20 July 1993 when six racks were deployed in the forebay and ended when three racks were removed on each of 27 July and 30 July.

Recruitment preference data were analyzed by Friedman's test, which is appropriate for nonparametric randomized block analyses of variance (ANOVAs). Tukey's procedure was used for multiple comparisons of the data. All statistical tests were carried out using SYSTAT (Wilkinson 1990). Critical values for Friedman's test and Tukey's procedure were taken from Zar (1984). A probability level of $p < 0.025$ was considered significant.

Results

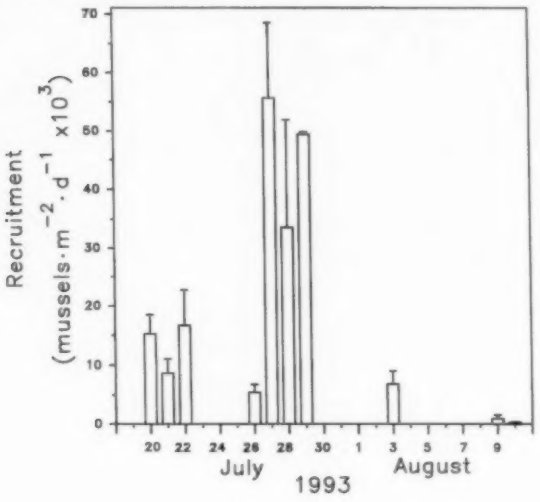
Total dreissenid recruitment

Mussel recruitment was not detected on acrylic plates that had been deployed in the forebay of Nanticoke T.G.S. 4 weeks prior to the experiment then removed and inspected 1, 2, and 3 weeks prior to the beginning of the experiment. Recruitment was not detected on August 11, 12, or 13 so the experiments were terminated.

During our experiments, mean mussel recruitment on the six treatments (i.e., live mussels, mussel shells, and stones on acrylic plates either with biofilm or with biofilm removed) varied from a low of 283 ± 104 mussels·m^{-2}·day^{-1} (mean ± standard deviation, $n = 18$) on 10 August to a high of 55 695 ± 18 940 mussels·m^{-2}·day^{-1} ($n = 17$) on July 27 (Fig. 2). On the first day of the experiment, 20 July 1993, the average rate of recruitment for all treatments was 15 200 ± 3192 mussels·m^{-2}·day^{-1} ($n = 18$). The size of the newly settled mussels was 390 ± 51 μm ($n = 40$) on 20 July, 279 ± 42 μm ($n = 40$) on 29 July, and 363 ± 90 μm ($n = 40$) on 9 August.

Two sets of three racks containing all six experimental treatments were suspended in the forebay of Nanticoke T.G.S. on July 20 to measure medium-term recruitment rates. One set of three racks was removed after 7 days (20 July – 27 July) and one set was removed after 10 days (20 July – 30 July). The three racks left in the water for 7 days attracted a total of 15 568 mussels (86 489 mussels·m^{-2}) whereas the racks that were deployed for 10 days attracted 32 690 settlers (181 611 mussels·m^{-2}). These values yield average daily recruitment rates of 12 300 and 18 200 mussels·m^{-2}·day^{-1} for the 7- and 10-day experiments, respectively. During the extended settlement experiments, other experiments were underway that measured daily recruitment. During the 7-day experiment daily recruitment was measured five times whereas during the 10-day experiment daily recruitment was measured seven

Fig. 2. Total daily recruitment of dreissenids on acrylic plastic plates and experimental substrates on 10 dates between late July and early August 1993. Error bars indicate standard deviation of the mean (n = 17 or 18).



times. Average recruitment rates calculated from the daily recruitment experiments are estimated to be 20 300 ± 18 100 mussels·m^{-2}·day^{-1} (n = 5) during the 7-day experiment and 26 000 ± 18 600 mussels·m^{-2}·day^{-1} (n = 7) during the 10-day experiment. It was not possible to statistically compare recruitment measured daily with areal recruitment measured over an extended period, given the low number of replicates for the extended period data.

Substrate preference during dreissenid recruitment

The maximum daily rate of recruitment measured was 114 800 mussels·m^{-2}·day^{-1} (n = 3) on the treatment with mussel shells and intact biofilm on 28 July. A number of treatments, particularly those without mussel shell or biofilm, failed to attract any settlers, particularly near the end of the experiment. Mean daily mussel recruitment for the whole experiment ranged from a maximum of 29 300 mussels·m^{-2}·day^{-1} (n = 10) for the mussel shells with intact biofilm to 4640 mussels·m^{-2}·day^{-1} (n = 10) for stones with biofilm removed by scrubbing (Table 1).

In our experiments dreissenid recruitment was significantly greater in the treatments that included mussel shells or live mussels than in the control treatments with stones as a substrate ($p < 0.001$, Table 2). There was no significant difference between the number of veligers settling on mussel shells and the number settling on live mussels.

On average 10–20% fewer recruits were found on treatments where biofilm had been removed by scrubbing (Fig. 3A) than on the identical treatment where biofilm was intact (Fig. 3B). Any treatment with biofilm had significantly greater recruitment than its counterpart with biofilm removed ($p < 0.002$, Table 2). Daily recruitment numbers varied over two orders of magnitude so a plot of fractional recruitment (recruitment on one treatment divided by total recruitment for that experiment) was used to illustrate surface preference in dreissenids (Fig. 3).

Table 1. Dreissenid recruitment (mussels·m^{-2}·day^{-1}) on acrylic plastic plates and substrates with either intact biofilm or biofilm removed by scrubbing with detergent.

Treatment	Substrate number	Average recruitment	SD	Min.	Max.
Mussels	1	18 900	21 300	300	83 700
Mussels + biofilm	2	24 300	27 800	400	113 200
Shells	3	18 400	20 600	0	84 100
Shells + biofilm	4	29 300	34 800	100	114 800
Stones + biofilm	5	13 900	18 400	0	55 600
Stones	6	4640	540	0	21 600

Note: The substrates were as follows: mussels (live, adult dreissenids), shells (dreissenid shells), or stones (compressed, oblong stones 1–3 cm in diameter). Sample sizes were 10.

Table 2. Statistical analysis of dreissenid recruitment on acrylic plastic plates and substrates with either intact biofilm or biofilm removed by scrubbing by detergent.

	Mussels	Mussels + biofilm	Shells	Shells + biofilm	Stones	Stones + biofilm
Mussels	—					
Mussels + biofilm	6.76*	—				
Shells	1.35	8.11*	—			
Shells + biofilm	5.16*	1.61	6.51*	—		
Stones	9.89*	16.65*	8.54*	15.04*	—	
Stones + biofilm	5.24*	12.00*	3.89	10.40*	4.65*	—

Note: The substrates were as follows: mussels (live adult dreissenids), shells (dreissenid shells), or stones (compressed, oblong stones 1–3 cm in diameter); each of these three substrates was a treatment with and without biofilm. Values reported are Friedman's χ^2_r.

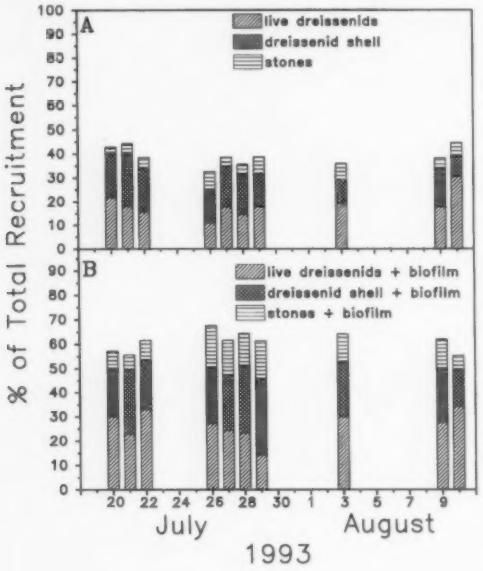
* Significantly different at $p < 0.025$.

Discussion

Our experiment appeared to encompass a complete dreissenid veliger recruitment period that lasted about 3 weeks, beginning in late July 1993. Recruitment began quickly and the most intense recruitment occurred near the middle of the settling period. No recruitment was apparent 1, 2, or 3 weeks before experiments began and recruitment had fallen to near zero by the end of our experiments. Recruitment rates were extremely variable; for example, on July 26 dreissenid recruitment was 5444 mussels·m^{-2}·day^{-1} but it was 55 694 mussels·m^{-2}·day^{-1} the next day.

Daily mussel recruitment rates appear to be lower when they are estimated from racks left out for 7 or 10 days. The daily settling rate estimated from racks left in the water for 7 consecutive days was 12 300 mussels·m^{-2}·day^{-1} whereas the average daily recruitment rate measured five times during the same 7-day period was 20 300 mussels·m^{-2}·day^{-1}. Similarly, daily recruitment estimated from racks left in the water for 10

Fig. 3. Substrate and biofilm preference in dreissenid larvae on 10 dates in July and August 1993. Recruitment was measured on acrylic plastic plates with live mussels, mussel shells, or stones with primary biofilm removed by scrubbing (A) and with the primary biofilm intact (B). Recruitment data are expressed as percentages of total daily recruitment.



consecutive days was 18 200 mussels·m^{-2}·day^{-1}, while the average recruitment rate measured seven times during the same 10-day period was 26 000 mussels·m^{-2}·day^{-1}. However, the standard deviation of the daily settling data is about 18 000 mussels·m^{-2}·day^{-1} so the difference may not be significant. If estimates of settling rate are lower when plates are deployed over longer periods of time it may be due to settled mussels detaching after death or migrating before the racks are collected (see Connell 1985 and Doka 1995 for further discussion of post-veliger mortality). It may also be that a well-colonized surface inhibits settling.

The average shell length of our newly recruited dreissenids ranged from 279 to 390 μm. In Lake Erie in 1992 Martel et al. (1994) found that the average length of newly settled dreissenids was about 250 μm, so the average length of ours is 10–55% greater than that of the 1992 dreissenids. Our length data are consistent with the settlement of quagga mussel veligers, which are more than 20% longer than zebra mussels at settlement (Martel 1995). Mussels on racks of acrylic plates deployed before there was any settlement in 1993 and removed in January 1994 at the same site as our experiments appeared to be exclusively quagga mussels (W.T. Claxton, University of Guelph, Guelph, ON N1G 2W1, unpublished data). These observations of increased length at recruitment and the high density of quagga mussels recruited to substrates in the forebay of Nanticoke T.G.S. are consistent with the general takeover of habitat in eastern Lake Erie in 1993 by quagga mussels.

Our measurements of daily areal recruitment rates in 1993 are difficult to compare with measurements from 1992 by Martel et al. (1994), since the interannual difference in potential rates of recruitment, which is influenced by a number of factors including veliger numbers, may be large. Also, Martel et al. (1994) did not measure recruitment by area but rather recruitment per 12 × 11 cm "scouring pad" per day. Assuming that the scouring pad collector is simply a flat surface of 264 cm^2 and that entrapment of veligers by the tortuous net of scouring pad fibres was minimal, then recruitment values for the data from Martel et al. (1994) range from 0 to 95 300 mussels·m^{-2}·day^{-1}. The upper limit of this range from Martel et al. (1994) is lower than what we measured for a preferred surface, such as shells with intact biofilm, but greater than what we measured for an unfavoured surface, such as stones with biofilm removed, so the scouring pad sampler is intermediate in its attractiveness to veligers. From a technical standpoint, however, there is little to recommend the scouring pad sampler because of possible entrapment of veligers and also because the scouring pad samples must be extracted, filtered, and preserved before being counted. Veligers settling on the plates used in this study were counted directly under the microscope without preservation.

We found that recruitment of dreissenids was intense and brief. In water that has been chlorinated to prevent mussel settlement in water intakes, settlement often begins with the appearance of large numbers of veliger larvae but it is weeks before significant recruitment occurs. Our work supports the contention of Claudi and Mackie (1994) that the duration of chlorination of raw lake water could be greatly decreased by focussing on the first signs of settling rather than on the first appearance of veligers, since the veligers themselves are not the problem. Martel et al. (1994) have shown that the appearance of late-stage veligers correlates well with recruitment, so either counts of late-stage veligers or direct measurements of recruitment could be used to synchronize chlorination with periods of recruitment and therefore decrease chlorine use.

We expected that late in the recruitment period veligers would settle indiscriminately on any available surface. However, in our experiments the preference of mussels for the substrates did not appear to change with time though we could not test directly for this phenomenon. We also expected that the discriminatory abilities of veligers would be less when large numbers were settling, but surface preferences appeared to be the same regardless of recruitment density (Fig. 3).

The presence of conspecific adults is known to be an important factor in the recruitment of many marine organisms. Investigations of barnacle larvae also suggest that surface bacterial films are important. Tamburri et al. (1992) found that both biofilm and conspecifics produce waterborne substances that are attractive to settlers. The results of our experiment support this observation. The amount of recruitment on live, adult mussels, shell from dead mussels, or stones was significantly increased by the presence of a 4- to 6-day biofilm. Though the nature of the interaction between settlers and biofilm is not clear our data suggest that biofilm removal will decrease the rate of colonization. Maki et al. (1989) suggested that a critical characteristic of larval preference is surface free energy. Barnacles have high settlement on surfaces with high critical surface energies (i.e., hydrophilic and highly wettable surfaces like glass). Maki et al. (1989) also found that the adhesive strength was greatest on substrates with high surface energies. Bryozoans, however, prefer surfaces with low

critical surface energies (hydrophobic and poorly wettable; Roberts et al. 1991). Surface energy is also important in bacterial attachment and colonization because the adsorption of macromolecules to solid substrates alters surface charge and energy (Fletcher and Marshall 1982). The adsorption of different proteins to a surface affects bacterial attachment (Fletcher and Marshall 1982). The biofilm in our experiments was not well characterized but surfaces with substantial biofilm in general have high surface energies, which suggests that dreissenids prefer similar surfaces to barnacles.

The physical removal of biofilm is not likely to be a practical method to control dreissenid recruitment even though we observed significant decreases in settlement. Biofilm removal by scrubbing only decreased settling and many mussels attached to the cleaned surfaces. Also, significantly enhanced mussel recruitment was seen even though our biofilm was only cultured for 4–6 days. Control of recruitment by biofilm removal would therefore involve physical removal of biofilm at intervals less than 4 days, which would be expensive and impractical and only about a 10–20% decrease in settling could be anticipated.

Though physical biofilm removal may be impractical, chemical removal of biofilm may be a viable control strategy. Chemical removal of biofilm is likely to be more effective at discouraging recruitment than physical removal of biofilm because biofilm could be continually removed with antibiofilm agents. In our experiments biofilm would have begun to form as soon as the plates were lowered into the water and cleaned treatments therefore may have provided a suitable surface for recruitment soon after being deployed. Certainly comparisons of the biofouling potential of surfaces must consider the recruitment-enhancing nature of surface biofilm.

There was no difference between the amount of recruitment on live mussels and mussel shells if both surfaces had an intact biofilm, or between live mussels and mussel shells if both surfaces had their biofilm removed. These results indicate that some physical or chemical factor in the shell was significant in encouraging recruitment and that the adult conspecifics did not appear to be producing recruitment-mediating compounds. In addition, settlers preferred both shells and live mussels, with or without biofilm, to stones for recruitment.

The preference of veligers for dreissenid shells suggests that removing mussels without removing all shells and associated material is an inadequate means for controlling settling and subsequent biofouling. To decrease recolonization all shells and associated material must be removed.

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Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*)

J. Kevin Craig, Chris J. Foote, and Chris C. Wood

Abstract: Temperature-dependent sex determination has been demonstrated in several animals, but in salmonids sex is generally believed to be under strict genetic control. We observed distorted sex ratios, attributable to a temperature manipulation during embryonic development, in experiments conducted during 1991 with the anadromous and nonanadromous (kokanee) forms of sockeye salmon (*Oncorhynchus nerka*) from Takla Lake, British Columbia. Sex ratios ranged from 62 to 84% female, and the biased ratio could not be accounted for by differential mortality by sex. The effect was observed independently in sockeye and kokanee crosses, as well as in the reciprocal hybrids. Similar crosses from Takla Lake in a previous year (1989) in which incubation temperature was not manipulated resulted in normal (1:1) sex ratios. Other populations of sockeye and kokanee from the same year (1991) but undergoing no temperature manipulation maintained normal sex ratios, as did populations from several disparate locations and years (1986–1994). The parsimonious conclusion is that the temperature manipulation during development was responsible for the biased sex ratio through a direct influence on sex differentiation. Hence, the possibility that temperature-dependent sex determination occurs in *O. nerka*, and perhaps other salmonids, deserves rigorous testing.

Résumé : Chez plusieurs animaux, on a montré que la détermination du sexe peut être dépendante de la température, mais on considérait généralement que, chez les salmonidés, elle était régie par un strict contrôle génétique. Nous avons toutefois observé des distorsions du rapport des sexes, attribuables à une manipulation de la température pendant le développement embryonnaire, dans des expériences menées en 1991 sur les formes anadromes et non anadromes (kokani) de saumon rouge (*Oncorhynchus nerka*) du lac Takla, en Colombie-Britannique. Le rapport des sexes était de 62 à 84% en faveur des femelles, et cette distorsion ne pouvait pas s'expliquer par des différences dans la mortalité selon le sexe. L'effet a été observé de façon indépendante chez des croisements de saumon rouge et de kokani, ainsi que chez des hybrides réciproques. Des croisements semblables du lac Takla d'une année antérieure (1989) pendant laquelle la température d'incubation n'avait pas été manipulée a donné des rapports des sexes normaux (1:1). Chez d'autres populations de saumon rouge et de kokani de la même année (1991), mais n'ayant pas subi de manipulation thermique, les rapports des sexes étaient normaux, tout comme chez des populations provenant d'endroits divers et d'années différentes (1986–1994). Nous pouvons conclure avec circonspection que la manipulation de la température pendant le développement est responsable de la distorsion du rapport des sexes par influence directe sur la différenciation sexuelle. La possibilité que la détermination du sexe soit dépendante de la température chez *O. nerka*, et peut-être chez d'autres salmonidés, mérite donc une vérification rigoureuse.
[Traduit par la Rédaction]

Introduction

Environmental sex determination (ESD) is said to occur when gender is irreversibly influenced by the environment during development. ESD has been demonstrated in several invertebrates (Charnov and Bull 1977; Charnov et al. 1978, 1981; Naylor 1988), reptiles (reviewed in Deeming and Ferguson 1988), and a few fish species (Harrington 1968; Conover and Kynard 1981; Rubin 1985; Sullivan and Schultz 1986). However, ESD may be a more widespread mode of sex

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determination than previously thought (Conover et al. 1992) given that an adaptive function of ESD has been proposed (Charnov and Bull 1977) and elucidated in at least one fish (Atlantic silverside, *Menidia menidia*; Conover 1984). The most commonly identified environmental variable influencing sex is temperature (Bull 1983), though effects of pH (Rubin 1985), artificial treatment with hormones (Yamamoto 1969; Hunter and Donaldson 1983), and pollutants (Torblaa and Westman 1980) on sex ratio have been reported.

Phenotypic sex in salmonids (family: Salmonidae) is generally believed to be strictly under genetic control, though the actual mechanism by which sex is determined has not been adequately investigated. Some populations of sockeye salmon, *Oncorhynchus nerka*, have morphologically distinct sex chromosomes (Thorgaard 1978; Ueda and Ojima 1984), as do some populations of rainbow trout, *Oncorhynchus mykiss* (Thorgaard 1977, 1983). However, observations of individuals of atypical karyotype or phenotypic sex have been attributed to possible environmental influences and (or) the effects of autosomal genes (Thorgaard 1977, 1983; Scheerer et al. 1991). The induction of sex reversal via hormone treatment during early developmental stages in most species of Pacific salmon, *Oncorhynchus* spp., suggests that sex is labile during this period (Hunter and Donaldson 1983), but the influence of external factors such as temperature on sex determination has not been rigorously investigated. Sex appears to be fixed after some early point in development as intersexes have not been reported in nature.

Sockeye salmon and kokanee are the anadromous and nonanadromous forms of *O. nerka*, respectively. They occur allopatrically and sympatrically, and even where sympatric they are genetically distinct populations. Genetic distinction has been demonstrated through a series of controlled breeding experiments on populations from British Columbia (Wood and Foote 1990, 1995; Taylor and Foote 1991; Foote et al. 1992) and biochemical genetic surveys throughout North America and Asia (Foote et al. 1989; Wood and Foote 1995; Taylor et al. 1995).

Throughout the prior breeding studies cited above, sex ratios were consistently 1:1. However, during breeding experiments in 1991 the sex ratios of some crosses shifted drastically, and this shift was associated with a change in incubation temperature during embryonic development. In this paper we describe the sex ratios of sockeye salmon and kokanee crosses from five disparate locations and three separate freshwater systems, and conclude that sex is thermolabile in *O. nerka*.

Methods

1991 Takla Lake population

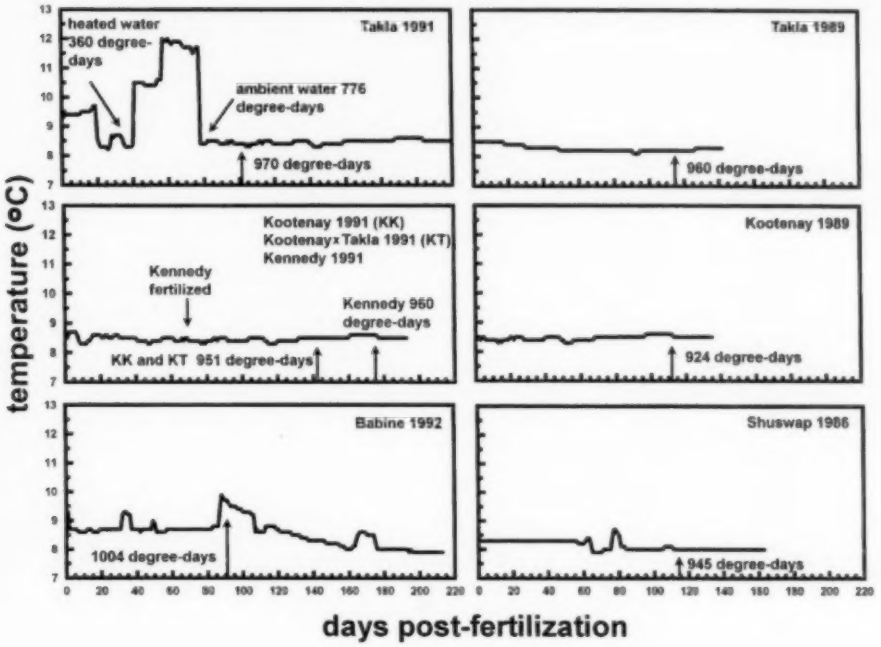
On 16 August 1991 random samples of ripe sockeye and kokanee were collected from Narrows Creek, Takla Lake, British Columbia, by beach seine. The eggs of each female were stripped into 1-L jars and fertilized. The jars were filled with chilled water and transported to a hatchery in coolers chilled to approximately 2–4°C. The genetic design consisted of a mixture of 43 pure and half sib families of pure sockeye (PS, 13 families), hybrid sockeye (HS; sockeye female × kokanee male,14 families), pure kokanee (PK, 9 families), and hybrid kokanee (HK; kokanee female × sockeye male, 7 families). A total of 43 males (20 sockeye and 23 kokanee) and 29 females (13 sockeye and 16 kokanee) were used to form the crosses.

Within 42–45 h of fertilization, the eggs were transferred to Heath tray baskets, surface disinfected with iodine solution, and incubated in 9.0 ± 0.8°C (mean ± SD) well water. Mortality was recorded at the eyed egg stage (18 September), dead eggs were removed, and the remaining live eggs were grouped in trays at a maximum density of 200 eggs. Temperature was recorded twice daily with an internal temperature probe. On 25 September (360 degree-days) the incubation temperature was deliberately manipulated in an effort to increase development rate and reduce incubation time (Fig. 1). The trays were moved from stacks supplied with ambient well water (8.3–9.7°C) to those supplied with heated well water (10.4–12.0°C) while covered to maintain light levels. On 2 November (776 degree-days) the trays were moved back to the ambient well water (8.3–8.5°C) in a similar manner. On 22 November (970 degree-days) all families were transferred as alevins ("ponded") into separate 40-L Calaprice tanks supplied with ambient well water at a density of approximately 200 fry/tank (range 74–214).

Manipulations after ponding reflect the requirements of various experiments and space limitations and probably did not affect sex ratios. On 23 January 1992 each family was randomly culled to a standard density (60 fish/tank). On 18 April alternate families were marked by clipping adipose fins so that consecutive clipped and unclipped families could be pooled into 480-L tanks (each tank contained two families of 57 fish each). On 27 July approximately 800 fish were individually PIT tagged (passive integrator transponder, Prentice et al. 1987) and moved to four 1500-L tanks. Each tank was identical in family composition, and each family was represented by an equal number of fish (203 fish/tank). The remaining fish were fin clipped to identify cross type, coded wire tagged (CWT, Bilton et al. 1982) to identify family, and reared in 480-L tanks at a density of 109 fish/tank. On 10 November all CWT fish were moved outdoors to two concrete channels (17 000 L) and one 2800-L tank. The channels contained fish of each of the four cross types, with families of each type split equally between the two channels. No HK were placed in the 2800-L tank because of their low numbers. The fish in the 2800-L tank were sampled monthly from January to July 1993. PIT-tagged fish were reared in indoor tanks for the duration of the experiment.

All fish were reared on a natural photoperiod for latitude 49°N. Dissolved oxygen (DO) was monitored at periodic intervals and flow manually regulated to ensure DO remained above 6 ppm. Fish were fed Biodiet starter mix, Hatchery diet, Promoter I diet (Whitecrest Mills), and Promoter II diet (Whitecrest Mills) at specific times during the 2-year period. Feed was dispensed by automatic feeders every half hour during daylight at a daily rate of 3.8–1.0% body weight per fish, decreasing progressively over time. Fish reared in indoor tanks were fed the same diets but under a variety of feeding regimes for a growth rate experiment. Mortality was very low during the experiments and was recorded throughout the 2-year period. Terminal sampling took place from 14 September to 6 October 1993. Sex was determined by visual inspection of the gonads.

Fig. 1. Daily incubation temperature during development for sockeye and kokanee (*Oncorhynchus nerka*) crosses from several populations throughout British Columbia. Takla Lake 1991 crosses underwent a temperature manipulation in which trays were moved to heated water on day 41 (8.4–10.5°C) and back to ambient water on day 78 (11.7–8.4°C). All other populations were reared in ambient well water. Arrows along the *x* axes indicate time of transfer ("ponding") of alevins from trays to rearing tanks maintained on the same ambient well water. Fertilization and ponding dates for Kennedy 1991 sockeye are shown relative to those of Kootenay kokanee and Kootenay × Takla "hybrid" kokanee from 1991.



Control populations

Several additional populations in which no temperature manipulations occurred have been reared under near identical conditions in the same hatchery since 1986. In 1989 pure and reciprocal hybrid crosses between sockeye and kokanee (five families each of PS and HS, eight families of PK) were generated with fish from Narrows Creek, Takla Lake (see Wood and Foote 1995 for details of rearing methodology). These crosses serve as a partial control for possible population and cross type effects, assuming that there was no year effect. In 1991 crosses were also made with fish from several additional populations from throughout British Columbia, and these served as a partial control for possible year effects. These fish were reared under the same conditions as the Takla 1991 and 1989 crosses but differed from the Takla 1991 crosses in that they experienced no shift in incubation temperature. The additional populations consisted of (*i*) 1991 pure kokanee crosses (10 families) from Kootenay Lake, British Columbia, on the Columbia River system, (*ii*) "hybrids" (4 families) between Kootenay Lake and Takla Lake (Fraser River system) kokanee (Kootenay Lake female from the wild × Takla Lake male from the 1989 Takla crosses), and (*iii*) pure sockeye crosses (20 families) from Kennedy Lake on Vancouver Island, British Columbia.

Sex ratio data were also available for pure kokanee crosses (12 families) from Kootenay Lake in 1989 (see Foote et al. 1994 for rearing methodology). These fish were reared in a similar manner to the 1991 Kootenay crosses, serving as a between-year comparison (1989 and 1991) of the same population undergoing no temperature manipulation. In addition, these were the same years as the between-year comparison within Takla Lake, in which 1991 fish experienced a temperature manipulation and 1989 fish did not. Also, sex ratio data were available for pure and reciprocal hybrid crosses of sockeye and kokanee (12 families of each of the four cross types) from Shuswap Lake in 1986 (see Foote et al. 1992 for rearing methodology), and pure sockeye crosses (7 families) from Babine Lake in 1992.

Complete sex ratio data were available for all of these populations with the exception of the Babine Lake population, which represented a random sample of fish taken on 3 October 1994 at 2 years of age. Though detailed descriptions of rearing methodology are not presented, these populations were maintained under standard hatchery conditions and sampled under a variety of protocols. Hatchery conditions (such as density, diet, and photoperiod) were remarkably constant across years and rearing temperature was consistently between 8 and 10°C (Fig. 1).

Table 1. Percentage of female progeny from crosses of sockeye salmon and kokanee (*Oncorhynchus nerka*) from Takla Lake, 1991.

Population	Brood year	Rearing protocol	*n*	PK	*n*	HK	*n*	HS	*n*	PS
Takla	1991	Outdoor channels	127	78.0*** (69.7–84.8)	132	82.6*** (75.0–88.6)	394	74.9*** (70.3–79.1)	288	61.8** (55.9–67.4)
		Indoor tanks	148	76.2*** (65.8–80.5)	127	81.1*** (73.2–87.5)	111	82.9*** (74.6–89.4)	199	70.4*** (63.5–76.6)
		Outdoor tank	56	83.9*** (71.7–92.4)	0	—	58	74.1* (61.0–84.7)	57	70.2* (56.6–81.6)
		Total	330	78.2*** (73.3–82.5)	259	81.9*** (76.6–86.4)	563	76.4*** (72.6–79.8)	544	65.8*** (61.7–70.0)
Takla	1989		98	54.1 ns (43.7–64.2)	35	54.3 ns (36.6–71.2)	135	45.2 ns (36.6–54.0)	78	60.3 ns (48.5–71.2)
Kennedy	1991		0	—	0	—	0	—	552	56.6 ns (51.5–60.0)
Kootenay	1991		152	39.8 ns (31.0–47.0)	0	—	0	—	0	—
Kootenay	1989		209	53.1 ns (46.1–60.0)	0	—	0	—	0	—
Kootenay × Takla[a]	1991		158	54.4 ns (46.3–62.4)	0	—	0	—	0	—
Babine	1992		0	—	0	—	0	—	419	48.2 ns (43.3–53.1)
Shuswap	1986		93	44.1 ns (33.8–54.8)	121	44.6 ns (35.6–53.9)	128	40.6 ns (32.0–49.7)	95	50.5 ns (40.1–60.9)

Note: PK, pure kokanee; HK, hybrid kokanee (kokanee female × sockeye male); HS, hybrid sockeye (sockeye female × kokanee male); PS, pure sockeye. Results of χ^2 analysis for deviation from a sex ratio of 1:1 are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, nonsignificant). Binomial confidence limits are given in parentheses.

[a]Hybrids between Kootenay Lake female kokanee from the wild (1991 brood) and Takla Lake male kokanee maturing in the hatchery (1989 brood).

Table 2. Mortality from 16 August 1991 to 6 October 1993 and adjusted sex ratio (% female assuming all male mortality) by cross type for Takla 1991 crosses.

Cross type	Number of embryos	Number of mortalities[a]	Percent mortality	Percent female
PK	1158	57	4.9	66.7*** (61.7–71.3)
HK	849	101	11.9	58.9* (53.6–64.0)
HS	5164	93	1.8	65.5*** (61.8–69.2)
PS	3887	49	1.3	60.4*** (56.3–64.3)

Note: PK, pure kokanee; HK, hybrid kokanee (kokanee female × sockeye male); HS, hybrid sockeye (sockeye female × kokanee male); PS, pure sockeye. Results of χ^2 analysis for deviation from a sex ratio of 1:1 are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, nonsignificant). Binomial confidence limits are given in parentheses.

[a]Includes any unfertilized eggs.

Data analysis

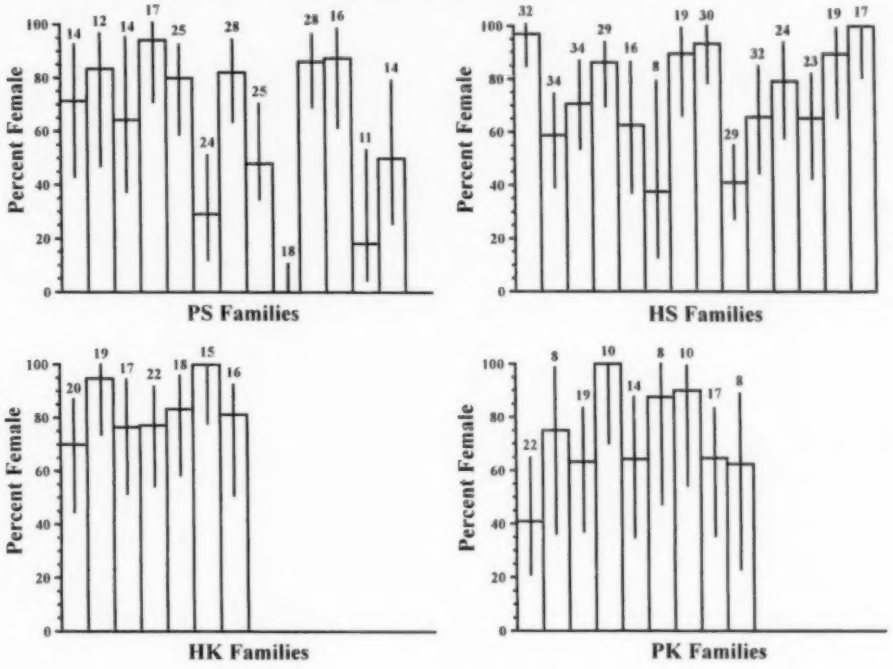
All fish were initially pooled by cross type and sex ratios were individually tested within each cross type for deviations from 1:1 by χ^2 analysis with Yates continuity correction (Zar 1984). Heterogeneity χ^2 analysis was used to examine differences in sex ratio among the three rearing protocols (indoor tanks, outdoor channels, outdoor tank sampled periodically during 1993) and the four cross types (PK, HK, HS, PS) from the Takla 1991 brood. This is a conservative analysis as deviations from a 1:1 sex ratio were tested repeatedly within each rearing protocol and cross type before pooling (see Results). Family sex ratio data were available for the four cross types from the Takla 1991 brood reared in the outdoor channels. χ^2 analysis was used to examine deviations from a 1:1 sex ratio within each family as well as variation in sex ratio among families. Fish of unknown sex accounted for a minor proportion of the data set (9 of 1696 individuals for Takla 1991 crosses; 0.5%) and were conservatively assumed to be males.

Results

Sex ratios shifted drastically in 1991 sockeye and kokanee crosses from Takla Lake (Table 1). Across the four cross types and rearing locations, sex ratios ranged from 61.8 to 83.9% female (all $P < 0.03$). Within a particular cross type, there was no difference in sex ratio among fish reared in outdoor channels, indoor tanks, or those sampled periodically during 1993 (Table 1, all $P > 0.35$). Therefore, data were pooled over the three sampling protocols. Even when all mortalities were combined with the males, a female-biased sex ratio remained (Table 2, all $P < 0.03$).

While sex ratios were similar among the three rearing protocols, the four cross types differed in sex ratio ($P < 0.02$). HK

Fig. 2. Sex ratio by family for pure sockeye (PS), hybrid sockeye (HS; sockeye female × kokanee male), hybrid kokanee (HK; kokanee female × sockeye male), and pure kokanee (PK) crosses from Takla Lake in 1991. Numbers above binomial confidence limits represent family sample sizes.



(81.9% female), PK (77.0% female), and HS (76.4% female) crosses had similar sex ratios,while PS had a less skewed sex ratio (65.8% female). This pattern was mirrored at the family level with female-biased sex ratios in 100% of HK, 89% of PK, 86% of HS, and 77% of PS families (Fig. 2). Thirty-five of the 43 families (79.1%) exhibited female-biased sex ratios (58.0–100.0% female, $P < 0.01$; Fig. 2), though only 15 were individually significantly different from 1:1 ($P < 0.05$). Further, families within cross type were significantly heterogenous in sex ratio for PS ($P < 0.001$) but not the other three cross types (all $P > 0.4$).

Populations reared under nearly identical conditions but undergoing no temperature manipulation exhibited balanced sex ratios (all $P > 0.05$; Table 1). Indeed, pure and reciprocal hybrid crosses from Narrows Creek, Takla Lake, in 1989 exhibited sex ratios that did not differ from 1:1 (all $P > 0.2$). PK from Kootenay Lake in 1989 and 1991, the same 2 years in which Takla Lake fish were reared, maintained sex ratios of 1:1 ($P = 0.58$ and 0.06, respectively; Table 1). In addition, hybrids between Takla Lake kokanee and Kootenay Lake kokanee, as well as pure sockeye from Kennedy Lake, generated in 1991 maintained sex ratios of 1:1 ($P = 0.56$ and 0.06, respectively; Table 1). Further, sockeye and kokanee crosses maintained under standard hatchery conditions from Babine and Shuswap lakes, British Columbia, two separate freshwater systems (Skeena and Fraser rivers, respectively), exhibited normal sex ratios ($P = 0.63$ and all $P > 0.15$, respectively; Table 1).

Discussion

We observed strongly female-biased sex ratios in crosses of sockeye and kokanee from Takla Lake in 1991. The female-biased sex ratio was observed in each of the four cross types and in the majority of families, despite the fact that sockeye and kokanee from Takla Lake are genetically distinct populations (Foote et al. 1989). Therefore, the result was replicated not only across families but across genetically independent populations and their hybrids. The consistency of sex ratios across environments and sampling protocols suggests that sex was irreversibly determined at some common point during early development.

Incubation temperature is probably the factor responsible for the shift in sex ratio. Populations reared under nearly identical conditions but undergoing no temperature manipulation exhibited balanced sex ratios. In addition, similar crosses from Takla Lake in 1989 exhibited balanced sex ratios. The parents of these crosses were collected at the same creek location and nearly the identical date (August 14) as in 1991, indicating that the female-biased sex ratio of 1991 Takla fish was not the result of a population or cross type effect. Further, PK from Kootenay Lake reared in the same 2 years as the Takla crosses but undergoing no temperature manipulation exhibited a normal sex ratio, strongly suggesting that the biased ratio of 1991 Takla fish was the result of the temperature manipulation. That sockeye and kokanee crosses from five disparate locations and three distinct freshwater systems (Fraser, Columbia, and

Skeena rivers) maintained sex ratios of 1:1 with the exception of pure sockeye and kokanee crosses (and their hybrids) undergoing a temperature manipulation, strongly suggests that temperature was the factor responsible for the shift in sex ratio of Takla 1991 crosses.

Other factors that might result in the skewed sex ratio of 1991 Takla fish can largely be ruled out. Culling was random with respect to sex and mortality was very low at all stages. Since a female-biased sex ratio remained after all mortalities were assumed to be males, the biased ratio could not be the result of differential mortality by sex. Although a control independent of temperature is not available for the actual movement of trays to and from the heated water, it is unlikely that slight mechanical agitation significantly affected the sex ratio as all control populations were subject to mechanical agitation at a similar stage to identify dead eggs. In addition, all embryos had hatched and alevins were mobile by the time trays were moved back to ambient water. Other environmental factors that might be responsible for the shift (photoperiod, dissolved oxygen, pH, contaminants) were either invariant or similiar among populations and years. As the hatchery is supplied by groundwater, contamination from surface pollutants (e.g., environmental estrogens) is unlikely and would have affected the other 1991 populations as well. Given the consistency of hatchery conditions across years, the magnitude of the shift in sex ratio, the replication of the effect across genetically distinct populations, the low mortality observed, and evidence for temperature effects on sex determination in other fishes (Harrington 1968; Conover and Kynard 1981), the temperature manipulation remains the most plausible cause of sex ratio distortion observed in the Takla 1991 crosses.

It is not clear what aspect(s) of the temperature manipulation was (were) responsible for the shift in sex ratio. The effect could have resulted from the the shift to an elevated incubation temperature (8.4–10.5°C), the actual incubation temperature itself (range 10.5–12.0°C) following the shift, and (or) the shift back to water of ambient temperature (11.7–8.4°C) (Fig. 1). It would appear unlikely that incubation temperature per se was responsible. Incubation temperature undoubtedly varies among hatcheries (see Table 7, Burgner 1991) and though few studies report sex ratios, it is improbable that such a strongly biased ratio would go unreported or unnoticed. It is more probable that the shift itself occurred during a critical point in development, inhibiting the phenotypic expression of maleness. During the sexual development of many fishes the gonads develop first as female-like ovaries and subsequently a proportion (usually 50%) further develop as testes (Yamazaki 1983). Perhaps the underlying physiological process(es) responsible for male development was (were) inhibited by the temperature shift. Embryos underwent an immediate temperature increase from 8.4 to 10.5°C at 360 degree-days followed by a decrease from 11.7 to 8.4°C at 776 degree-days. Sex determination may be sensitive to temperature within a developmental window during this period.

The four cross types differed in sex ratio, with PS exhibiting a less biased ratio than the other cross types. As sockeye and kokanee differ in development rate owing to both male and female genetic effects (Wood and Foote 1990), the cross types may have been at slightly different developmental stages when the temperature shift occurred. Alternatively, sockeye and kokanee may be polymorphic for labile sex determination such that they differ in sensitivity to temperature and (or) have different developmental windows during which temperature exerts an effect.

The majority of families (79.1%) of the four cross types had female-biased sex ratios though many were not significantly different from 1:1, probably because of small family sample sizes (8–40 fish/family). In addition, a small proportion of families were male biased, some extremely so (Fig. 2). For example, one PS family contained all males ($n = 32$) whereas two other families were strongly male biased (70.8 and 81.8% male; $n = 24$ and 11, respectively). That family sex ratios were either strongly male or female biased with few families exhibiting sex ratios approaching 1:1 suggests that a temperature-dependent, major sex determining gene may be segregating in the population (see Lagomarsino and Conover 1993). The existence of such a gene could account for the heterogeneous sex ratios observed among PS families.

Some populations of sockeye and kokanee exhibit morphologically distinct sex chromosomes (sockeye, Thorgaard 1978; kokanee, Udea and Ojima 1984). Sockeye and kokanee from Takla Lake have not been cytologically examined. However, assuming dimorphic sex chromosomes are present in this population as well, presumably some of the females from the Takla 1991 brood had XY sex chromosomes. Therefore, XY females when mated to XY males could give rise to XX, XY, or YY offspring, even under normal rearing conditions.

It is not clear whether ESD may be adaptive in *O. nerka* or, alternatively, some genetically determined, physiological process was simply inhibited in our experiments by temperature shock. Conover and Kynard (1981) demonstrated that higher rearing temperatures result in a greater proportion of males in silversides and the magnitude of this response varies latitudinally among allopatric populations (Lagomarsino and Conover 1993). In addition, ESD in this species appears to be adaptive (Conover 1984) and under the control of major and minor sex determining genes as well as temperature (Conover and Heins 1987; Conover et al. 1992; Lagomarsino and Conover 1993). Adaptive explanations also have been postulated for ESD in reptiles (Deeming and Ferguson 1988; Janzen and Paukstis 1991).

That a species with morphologically distinct sex chromosomes (Thorgaard 1978; Ueda and Ojima 1984) appears labile for sex determination is intriguing. Given the growing recognition of the effects of environmental factors on sex determination, the possible effects of global climate change and human-induced temperature shifts in localized environments, and the economic importance of salmonid aquaculture, the possibility of temperature-dependent sex determination in salmonids deserves further investigation.

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Fixed exploitation rate strategies for coping with effects of climate change

Carl Walters and Ana M. Parma

Abstract: Survival rates and carrying capacities for larval and juvenile fishes may be strongly affected by long-term, unpredictable climatic fluctuations. When climate impacts produce strongly autocorrelated interannual variations in recruitment, harvesting a constant fraction of the stock each year allows the spawning stock to track such variations. Dynamic programming analysis indicates that this tracking effect is likely to produce long-term harvests that are very close (within 15%) to the theoretical optimum that could be achieved if all future climatic variations were known in advance. Fixed harvest rate strategies are likely to degrade performance more than 10% only when there is little interannual correlation in environmental effects or when there is a large, abrupt climate change that can be predicted well in advance if it is going to increase carrying capacity, or detected immediately if it causes a decrease in capacity. This finding implies that it may be more cost effective to invest in research on how to implement fixed harvest rate strategies than to invest in research on explaining and predicting climatic effects. Successful implementation may require a combination of improved stock size assessments, and stringent regulatory measures to substantially restrict the proportion of fish at risk to fishing each year.

Résumé : Les taux de survie des larves et des juvéniles de poissons et la capacité portante de leurs habitats peuvent être profondément affectés par des fluctuations climatiques imprévisibles et à long terme. Quand l'impact du climat produit des variations interannuelles du recrutement qui sont fortement autocorrélées, le prélèvement d'une fraction constante de la population chaque année permet au stock reproducteur de s'aligner sur ces variations. L'analyse par programmation dynamique indique que cet effet d'alignement doit vraisemblablement produire à long terme des prélèvements qui sont très proches (en-deçà de 15%) de l'optimum théorique qui pourrait être atteint si on connaissait d'avance toutes les variations climatiques futures. Les stratégies du taux fixe de prélèvement ne réduisent la production de plus de 10% que lorsqu'il y a peu de corrélation interannuelle dans les effets environnementaux ou que se produit une modification climatique importante et brutale que l'on peut prévoir longtemps à l'avance si elle doit accroître la capacité portante, ou que l'on peut détecter immédiatement si elle cause une baisse de la capacité. Cette découverte permet de penser qu'il serait plus rentable d'investir dans la recherche sur les façons de mettre en oeuvre des stratégies de taux de prélèvement fixe plutôt que d'investir dans la recherche sur l'explication et la prédiction des effets climatiques. Pour mettre en oeuvre avec succès de telles stratégies, il peut être nécessaire de combiner des évaluations plus fiables de la taille des stocks et des mesures de réglementation stricte de façon à restreindre substantiellement la proportion de poissons susceptibles d'être pêchés chaque année.
[Traduit par la Rédaction]

Introduction

Long-term changes in marine and freshwater environments are one of the largest threats to fisheries sustainability. Such changes affect production (especially recruitment) directly and cause severe confusion in management systems when assessment scientists cannot distinguish between climatic and harvesting impacts. Probably every major fisheries decline has engendered fierce debate about whether environmental factors or fishing (or both) is the culprit, and this debate can lead to very damaging delays in corrective responses such as reduced allowable catches. There is little prospect that fisheries research will provide quick enough answers in such debates (Walters and Collie 1988), and therefore there is a critical need to develop management strategies that are robust to causes of fluctuation (provide near optimal long-term harvest no matter what the cause), are easily understood by all management stakeholders, are practical to implement, and avoid unnecessarily high variation in catches from year to year. The fixed exploitation rate strategy, where management aims to take the same proportion of the stock each year, is one popular possibility for meeting these criteria (Aron 1979; Deriso 1985; Hilborn 1986; Walters and Ludwig 1987; Hall et al. 1988; Hanneson 1989; Hilborn and Walters 1992; Pelletier and

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Laurec 1992; Mace 1994); criticisms of it have been mainly related to concerns about implementation when using inaccurate stock assessment data (Eggers 1993), especially where there is risk of depensatory effects (consistently high exploitation rates when stock size is low).

This paper compares the performance (total long-term catch) of fixed harvest rate strategies with the theoretical optimal performance that would be possible if all future environmental changes were known today. The comparison is done by generating simulated populations with complex, plausible patterns of recruitment variation, but using population dynamics models that are structurally simple enough to permit direct calculation (by dynamic programming) of the theoretical optimal performance. The analysis extends previous work by Parma (1990), who showed that optimal harvesting policies in nonstationary environments involve anticipatory adjustment of escapements (build up spawning stock before good conditions develop) to best capitalize on increased production opportunities. Results for cyclic environments indicate that such adjustments may lead to (*i*) only slight improvement over fixed harvest rate strategies that passively allow spawning stock to build up in response to favorable environmental changes, and (*ii*) greatly increased interannual catch variation. Parma speculated that cycles and other strongly autocorrelated patterns of environmental effects on recruitment would enhance the performance of fixed harvest rate strategies relative to that of the simpler fixed escapement strategy that is optimal when environmental effects are stationary and uncorrelated, adding to the variety of reasons that other authors (e.g., Hilborn and Walters 1992) have offered for preferring fixed harvest rate to fixed escapement strategies. Here we show that this speculation was indeed correct; we demonstrate that fixed harvest rate strategies, if implemented accurately, should generally result in long-term performance that is within 15% or so of the theoretical optimum, for a wide variety of populations; much lower performance is expected only in the case of extremely productive stocks (very high optimal harvest rates) with recruitments that are strongly influenced by uncorrelated environmental effects.

The following sections first derive a fairly general model for representing effects of environmental change on recruitment and population performance, then use this model to compare performances of fixed harvest rate and theoretical optimal policies. A final section discusses alternative approaches to implementation of fixed harvest rate strategies.

A model for environmental impacts on population dynamics

It is likely that future climate changes will affect mainly juvenile survival rates, and hence the recruitment process. Discussion of fixed harvest rate policies requires precise definition of how juvenile mortality rates may be influenced, since some types of effects have no consequence for the assessment of the best fixed harvest rate. Some discussions of effects of climate change on fisheries focus on notions of change in marine carrying capacity (e.g., Beamish and Bouillion 1993) while others emphasize physical changes that could influence either maximal recruitment rates or reproductive performance in depressed populations (or both, e.g., Mann 1992). We begin with the basic model suggested by Beverton and Holt (1957) that represents mortality during the juvenile stage as being linearly related to juvenile density at any moment:

$$[1] \qquad \frac{dN}{dt} = -MN$$

where $M = M_1 + M_2N$. Here M_1 represents density-independent mortality risk owing to processes such as larval advection from favorable rearing areas, while M_2 represents density-dependent risk related to juvenile crowding of available habitat (food, space) and possibly attraction of predators at higher juvenile densities. Obviously some environmental changes may simply kill more juveniles indiscriminately (increase M_1), while others may influence the basic habitat size over which juvenile interactions occur (influence M_2). The common observation that total recruitment is unrelated to egg numbers over a wide range of spawning densities requires that the density-dependent effect M_2 be quite large; when $M_2 = 0$, eq. 1 results in a basic prediction of recruitment proportional to egg production (N at time 0), not independent of egg production. Assuming that egg production is proportional to spawning biomass S_t (eggs = fS_t, where f is fecundity per unit spawning biomass), then integrating eq. 1 to an age at recruitment t_r results in the familiar Beverton–Holt recruitment model for recruits R_t in year t:

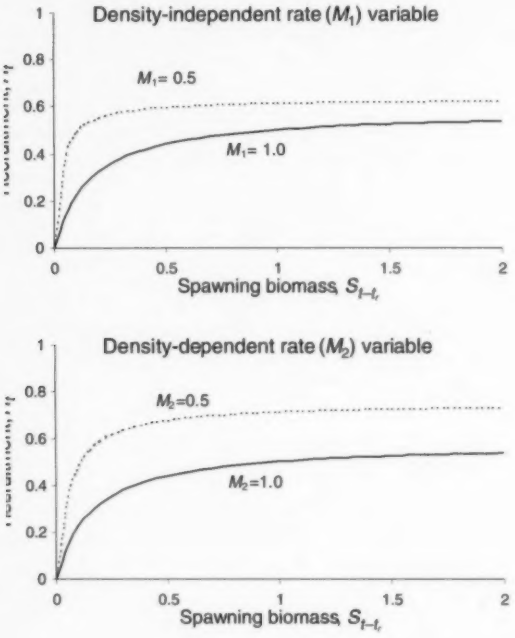
$$[2] \qquad R_t = \frac{S_{t-t_r} f e^{-M_1 t_r}}{1 + \frac{M_2}{M_1}(1 - e^{-M_1 t_r}) f S_{t-t_r}}$$

$$= \frac{AS_{t-t_r}}{1 + AS_{t-t_r}/K}$$

where $A = fe^{-M_1 t_r}$ and $K = M_1/(M_2(e^{M_1 t_r} - 1))$. Note here that changes in M_1 influence both A, the slope of the recruitment curve at low spawning abundances, and K, the asymptotic recruitment rate at high spawning abundance. Changes in M_2 are expected to influence only K. Thus we can model long-term, stochastic changes in factors that determine habitat size by treating only K as stochastic, whereas both A and K must be varied to represent stochastic changes in factors that influence the M_1 values of juveniles (Fig. 1). To avoid unnecessary complication in optimization calculations, we deal only with the case $t_r = 1$ (1 year lag to recruitment); see Parma (1990) for discussion of effects of longer delays on optimal policies.

To simulate long-term environmental effects on recruitment, we represented either M_1 (A–K variation) or M_2 (K variation) as an autoregressive random process of the form $M_{it} = \overline{M}_i + d_t$, where $d_t = rd_{t-1} + \omega_t$ has lag-1 autocorrelation $0 \le r < 1$ and independent, normally distributed changes ω_t with mean zero and variance σ^2. This model produces a total variance for the d_t of $\sigma^2/(1 - r^2)$. Thus, we could control the magnitude or violence of A and K changes by varying both r and σ^2, and the persistence of these changes by varying r (annual survival rate also influences persistence of effects on harvestable biomass; see below). When combined with growth–survival dynamics, the autoregressive process produces surprisingly realistic patterns of long-term population variation (Fig. 2), with periods of apparently cyclic behavior interspersed with periods of more erratic population change. For comparison of policy alternatives, it certainly produces perverse enough variation to

Fig. 1. Environmental factors may cause changes in both density-independent and density-dependent mortality rates of juvenile fish; changes in density-independent rates are predicted to affect both reproductive performance at low stock sizes and the "carrying capacity" maximal recruitment rate, while changes in density-dependent mortality rates are predicted to affect only the maximal recruitment rate.



challenge any decision rule for responding to or anticipating environmental change.

Growth–survival effects of population age structure were represented by incorporating the recruitment dynamics into a Deriso (1980) – Schnute (1985) delay-difference model of the form

$$[3] \quad B_{t+1} = g_t S_t + W_k R_{t+1}$$
$$S_t = B_t - C_t$$

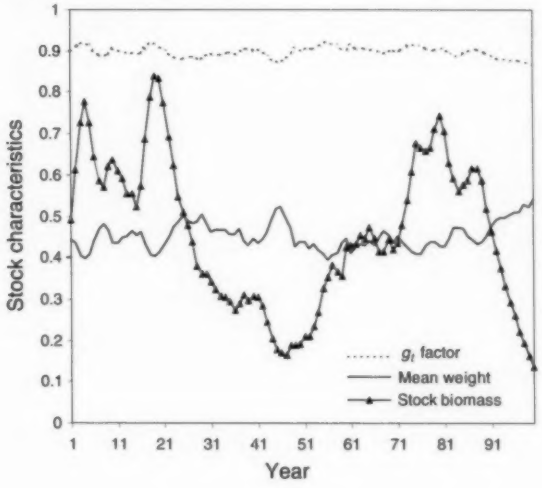
where B_t and C_t are stock biomass and catch in year t, W_k is body weight at entry to the harvestable population, and g_t is the growth–survival factor (Hilborn and Walters 1992).

$$[4] \quad g_t = s\left(\frac{\alpha}{\overline{W}_t} + \rho\right)$$

Here, s is the annual natural survival rate ($s = e^{-M}$), α and ρ are the Walford plot intercept and slope for weight at age against weight at the previous age, and $\overline{W}_t$ is the mean body weight of animals in the exploitable population in year t. We noticed in simulations (Fig. 2) that $\overline{W}_t$ and g_t are relatively stable provided the exploitation rate is also stable. Then, for any fixed exploitation rate u, $\overline{W}_t$ is near the value predicted by the equilibrium mean weight relationship

$$[5] \quad \overline{W}_e = \frac{s(1-u)\alpha + W_k(1-s(1-u))}{1-\rho s(1-u)}$$

Fig. 2. Simulation of long-term population changes using a delay-difference model and strongly autocorrelated deviations from long-term average recruitment relationship. Fluctuations are typical of simulations used to compare fixed harvest rate and optimal escapement policies in later figures; note the relatively small changes in average body size $\overline{W}_t$ and growth–survival factor g_t.



and $$g_t \equiv g_e = s\left(\frac{\alpha}{\overline{W}_e} + \rho\right)$$

Stability of g_t permits a dramatic simplification of optimization calculations for long-term yield; for the assessments below, we replaced g_t in eq. 3 with the equilibrium value g_e evaluated at u equal to the optimal exploitation rate, i.e., that value of u for which long-term yield is maximal.

The optimal long-term exploitation rate for the model defined by eqs. 2 and 3 can be estimated from the equilibrium relationship between B and S. The exploitation rate u_e that maximizes the average annual catch, $B - S$, for the deterministic case of eqs. 2 and 3 is given approximately by

$$[6] \quad u_e = 1 - \frac{1}{g_e + \sqrt{AW_k(1-g_e)}}$$

(This equation is solved iteratively: use a trial value of u_e to calculate g_e from eq. 5, use the resulting g_e to calculate an improved u_e from eq. 6; repeat until u_e stops changing.) Equation 6 is derived by maximizing the equilibrium catch with respect to u while treating g_e as constant (i.e., ignoring that g_e actually increases with u and in turn affects the catch). As a result, eq. 6 provides a lower bound to the true optimal exploitation rate. Simulation tests showed that this approximation gives very close to the maximal long-term yield obtainable from any fixed exploitation rate (even when stochastic variation is large), except that it tends to slightly underestimate the optimal u for very long-lived ($g_e > 0.9$) populations; in such cases the optimal u can be 10–20% higher than indicated by eq. 6, though the long-term yield corresponding to u_e will be within a few percent of the maximal long-term yield (the underestimation of u is compensated partly by the increase in the average biomass). Note that u_e increases with A, but it is

independent of the recruitment scaling or carrying capacity parameter K; this is characteristic of optimal equilibrium exploitation rate calculations for population dynamics models in general (the best relative harvest rate cannot depend on units of measurement of population size), and implies that u_e may give performance that is robust to variation in carrying capacity.

Suppose it were somehow possible to predict exactly future juvenile mortality rates (M_{1t} and M_{2t}) of each year $t = 1, ..., T$, for large T (e.g., 100 years), and to exactly assess the stock biomass B_t available in each of those years. Then the lucky manager would be able to calculate the catch to take each year so as to maximize the total catch over all t years, by dynamic programming value iteration. That is, solve the sequence of simple, deterministic maximization problems:

$$[7] \quad V_t(B_t) = \text{MAX}_{S_t \le B_t} (B_t - S_t + V_{t+1}(B_{t+1}(S_t, M_{it+1}))), \qquad t = T-1, T-2, ..., 1$$

where $B_{t+1}(S_t, M_{it+1})$ is next year's biomass predicted from eqs. 2 and 3 knowing incoming recruitment mortality. The backward iteration starts with boundary condition $V_T(B_T) = B_T$. Numerical solution of eq. 7 over a discretized grid of (B, S) values is easily done using methods described in Walters (1986), Hilborn and Walters (1992), and Mangel and Clark (1988); for calculations reported below, we used grids with 100 discrete levels of both B and S, and interpolated V_{t+1} linearly for B_{t+1} values between grid points. The solution is a sequence of time-varying optimal spawning escapements S^*_t, $t = 1 ... T$, which would take full advantage of environmentally driven changes in recruitment capacity K and productivity A.

Fixed escapement policies (S^* not dependent on t) are often reported to be optimal in harvest policy studies; they are in fact only best for maximizing long-term catch in cases where g_e is constant and M_{it} values are unknown but come from the same known, independent probability distribution each year ($r = 0$ above). In the results presented below, the S^*_t values differ from the absolute theoretical optimal values (given perfect future knowledge) for the model described by eqs. 2 and 3 only through trivial effects associated with numerical discretization, and with using $g_t = g_e$ in the dynamic programming calculation. For environments with strong, autocorrelated recruitment fluctuations as concern us, the calculated S^*_t values performed so much better than any fixed escapement alternative that we do not present any results for fixed escapement tests. However, note that simple fixed escapement policies can outperform fixed exploitation policies even in autocorrelated environmental situations, especially if there is risk of depensatory effects and associated low stock size equilibrium (Collie and Spencer 1993).

A "myopic" optimal escapement policy can be constructed by assuming that B_t and the most recent recruitment anomaly d_t are known exactly, but that future anomalies are unknown and only the probability distribution for anomaly changes is known (r, and the probability distribution of ω_t for all future t). In this case, dynamic programming formulation (replace V_{t+1} in eq. 7 with the expectation of V over the probability distribution of ω_t) indicates that the optimal policy will be a feedback function of (B_t, d_t). This feedback function can be expressed as an optimal escapement $S'(d_t)$ that varies with d_t (the optimal catch for year t is the surplus $B_t - S'$). Parma (1990) showed for models with fixed g_e that a tight lower bound for $S'(d_t)$ is given by the escapement level at which

$$E\left[\frac{dR_{t+1}}{dS_t}\right] = 1 - g_e$$

where the expectation is taken over possible recruitment anomalies in year $t + 1$ (here represented by d_{t+1}). When it is unlikely for B_{t+1} to be less than future possible myopic optima $S'(d_{t+1})$, then $S'(d_t)$ approaches the lower bound defined by the condition above. We tried some numerical solutions of this formulation for various A, g_e combinations, and found that S' values are very close to the S value that maximizes the equilibrium value of $B - S$ (average equilibrium catch), if B is calculated from eqs. 2 and 3 while holding d_{t+1} fixed at its expected value rd_t. That is, the myopic optimal policy is closely approximated by the results of deterministic optimization under the assumption that all future recruitment anomalies will equal the expected anomaly (if $\omega_t = 0$) for next year. This approximation is obtained by applying the condition above while setting $d_{t+1} = rd_t$; it thus results in escapements that increase linearly with anticipated carrying capacity at time $t + 1$, but may increase or decrease with changes in productivity A_t depending on the actual parameter values (see Mousalli and Hilborn 1986). Below we compare the expected harvest performance under the equilibrium approximation for myopic S' with both the theoretical best performance given by S^* and the performance using u_e.

Policy comparisons

Figure 3 illustrates how different the policy prescriptions can be from S^* (future known with certainty), S' (current environmental state known each year), and u_e (no knowledge of environmental change, just take same fraction of stock each year). For this example, we set $W_k = 1$ and held A in eq. 2 constant at a relatively low value implying $u_e = 0.1$; we set $g_e = 0.8$ (long-lived species), and varied K as shown (Fig. 3*a*) to produce a single period of years of very high recruitment carrying capacity. The numerical dynamic programming solution for S^* shows anticipatory behavior like that which Parma (1990) showed for cyclic recruitment situations; it is best to begin building up the spawning stock well in advance of a known increase in recruitment carrying capacity, so as to capitalize on the high potential production when it does arrive. In contrast, the myopic policy S' sees this opportunity only after it arrives, and shuts down the fishery just at the time when the optimal policy would begin to realize returns from the investment of increasing S before the environmental change. The fixed exploitation policy results in some benefit from the high K period (stock builds up during the period), but most of this benefit is lost when high harvests are not taken when the stock begins to collapse naturally (K falls back to low level). In this extreme case, the long-term (100 year) harvest for the perfect information policy is about 5% higher than that achieved by the myopic policy, and 15% higher than that produced by the fixed exploitation policy. The practical policies look much worse if evaluated over a shorter time period immediately surrounding the period of high carrying capacity.

Fixed exploitation and myopic policies are better at tracking the theoretical optimal escapement in simulated

Fig. 3. Comparison of spawning biomasses and catches over time under three harvesting strategies for a population that shows a single period of enhanced maximal recruitment rate (a); units are arbitrary. The theoretical optimum was calculated by dynamic programming assuming full knowledge each year of all future recruitment deviations; the myopic optimum was calculated by assuming that next year's deviation will be the same as this year's; the fixed exploitation strategy takes the long-term average best fraction of the stock each year. Note how the optimal policy shuts down fishing well in advance of an increase in carrying capacity, "anticipating" opportunity for producing more from larger spawning stocks after the increase; such anticipatory behavior is not found in optimal policies for very productive stocks that can build up quickly as the environmental change occurs.

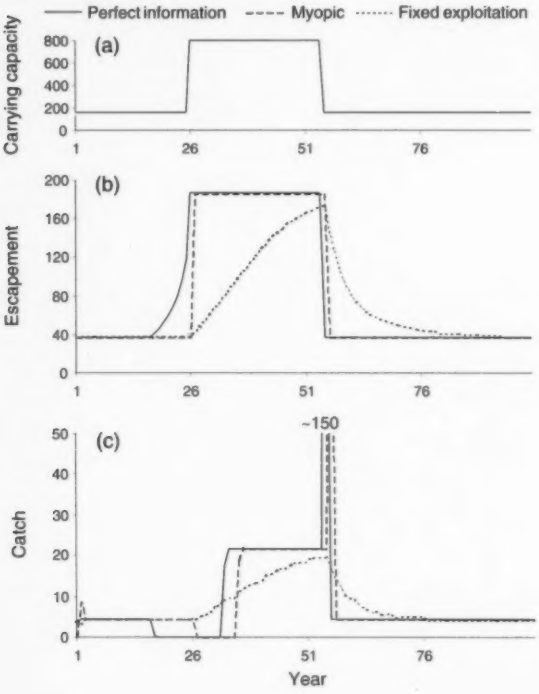
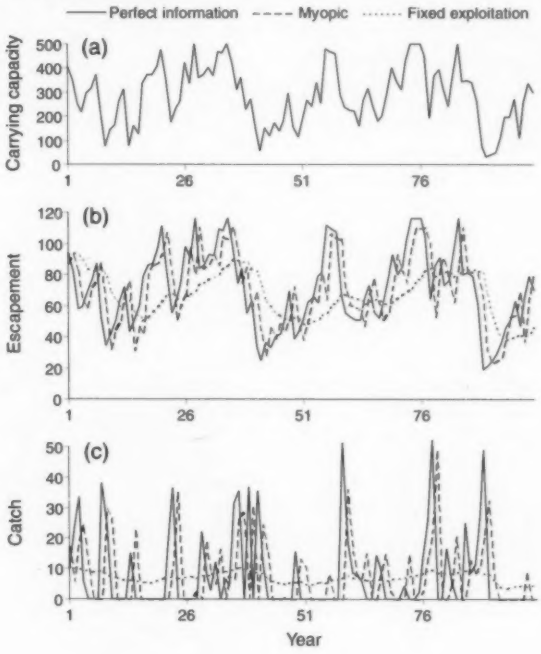


Fig. 4. Comparison of spawning biomasses and catches over time under three harvesting strategies, for a simulated "cod" population (long-lived, optimal harvest rate 0.1) with strongly autocorrelated environmental effects on maximal recruitment rate (a); units are arbitrary. As in Fig. 3, the theoretical optimum was calculated by dynamic programming assuming full knowledge each year of all future recruitment deviations; the myopic optimum was calculated by assuming that next year's deviation will be the same as this year's; the fixed exploitation strategy takes the long-term average best fraction of the stock each year.



situations where autocorrelated environmental effects produce less abrupt changes in the recruitment carrying capacity (Fig. 4). In such cases, the practical policies most often achieve at least 90% of the long-term perfect information harvest, with the myopic escapement policy generally performing better as it adjusts S_t much more quickly than would occur with a fixed exploitation rate. It is a bit surprising that the fixed exploitation rate policy does so well in spite of damping escapement variation and causing large response lags (like Fig. 4) in most simulation cases for long-lived (large g_e) species. Lags in policy response could be longer and potentially more harmful for longer lags to recruitment ($t_r >> 1$) if environmental conditions changed with a periodicity of about twice the mean age of reproduction. In such cases, trends in reproductive biomass would lag, and be out of phase with, the environmental trends affecting juvenile survival, and periods of high productivity would not be capitalized. The autoregressive processes used in our simulations generate trends in recruitment anomalies that tend to persist longer than common delays in the population dynamics, and so they can be more closely tracked by the stock.

Policy performance should depend on the source of stochastic variation in recruitment: density-independent (M_1) or density-dependent (M_2) parameters. In principle, variation in M_1 causes variation in u_e (through effects on A in eq. 6). However, a harvest rate equal to $u_e(M_1)$ would be the optimal fixed harvest rate only if M_1 remained constant in the future. But as M_1 continues to change, using the long-term mean $\overline{M}_1$ for calculating A and u_e generally works very well. Optimal escapement policies, on the other hand, are much more sensitive to changes in M_2 (K variation) than they are to changes in M_1 (A–K variation). Thus, environmental effects on the recruitment carrying capacity K appear to dominate simulated policy performance. The results below apply to the case where only K varies; variation in A as well does not change the basic conclusions.

Natural stochastic changes are unlikely to be as abrupt and persistent as in the case in Fig. 3, but they could vary

substantially in the frequency of occurrence of changes for which anticipatory variation in S^* (i.e., foresight about them) would really be worthwhile (i.e., a few simulation cases like Fig. 4 could be quite misleading). Therefore, we generated a large number of 100-year stochastic M_{it} sequences, computed the S^* and optimal catch sequence for each along with the S' and u_e catch sequences, and examined the resulting frequency distributions (over sequences) of S' and u_e relative performance. This tedious computational exercise allowed us to determine if autocorrelated environmental changes are at all likely to produce a recruitment anomaly sequence for which a fixed exploitation policy would perform very badly. Nature could of course be more perverse than any autocorrelation model, but variation much worse than shown in Figs. 2 and 4 (typical sequences from the simulation trials) seems unlikely to occur in most fisheries that are at all sustainable.

We generated 1000 M_{2t} sequences of 100 years for different types of populations ("salmon," "herring," and "cod") and autocorrelation levels r (0, 0.3, and 0.8). Figure 5 summarizes 9000 such simulations when the variance of ω_t was 0.05 (giving 2- to 3-fold variation in carrying capacity). The salmon example represents a productive, semelparous species with $g_e = 0$ and $AW_k = 4$ so $u_e = 0.5$. The herring example is a relatively short-lived species of intermediate productivity, with $g_e = 0.6$ and $AW_k = 1.35$ so $u_e = 0.25$. The cod example is long-lived and unproductive, with $g_e = 0.9$ and $AW_k = 0.45$ so $u_e = 0.1$. Biomass was initially set to the deterministic equilibrium for u_e and average environmental conditions. The most important determinant of relative performance for the practical policies was persistence of population response, as influenced by both g_e and r. The lowest relative performance was for the salmon case lacking population carry-over and environmental autocorrelation, but the practical policies do well even for this case when r is high. Performance was generally good (85% or better) for the long-lived cod case even when $r = 0$. The salmon case showed somewhat lower relative performance for fixed exploitation rates in the $r = 0$ situation than has been reported in previous comparisons of fixed exploitation versus fixed escapement policies (review in Hilborn and Walters 1992); this is because the comparison was not to a fixed escapement policy but rather to an optimal policy where full advantage was taken each salmon generation of a capability to predict stochastic survival for the next generation. The standard fixed escapement policy used in previous published comparisons corresponds here to the myopic policy, which resulted in yields about 15% higher on average than those attained by the fixed exploitation policy.

In nature r likely lies between 0.3 and 0.8, and for such situations a fixed exploitation rate policy is almost certain to provide at least 85% of the yield that can be obtained by any practical (not requiring environmental prediction) policy alternative (Fig. 5). Put another way, investment in developing means to predict environmental change (so as to allow S^* policies) would appear to be justified only for highly productive, short-lived species in environments that do not exhibit persistent change. But even in such extremes, we question the wisdom of implementing an S^* policy even if it were feasible; as shown in Fig. 3, such policies may maximize total harvest, but also come close to maximizing year-to-year variation in harvest and hence disruption of fishing industries (see Parma 1990 for a comparison between catch-maximizing policies for cyclic environments and policies that reduce variation in catches). The results above appear to hold for other types of stock-recruitment models, as long as long-term average environmental effects on density-independent sources of mortality are stable (mean of M_{1t} constant in our case); indeed, our results are consistent with those in Parma (1990), where a dome-shaped stock-recruitment function was assumed and environmental effects were modeled as cyclic trends in the density-independent mortality rate of juveniles.

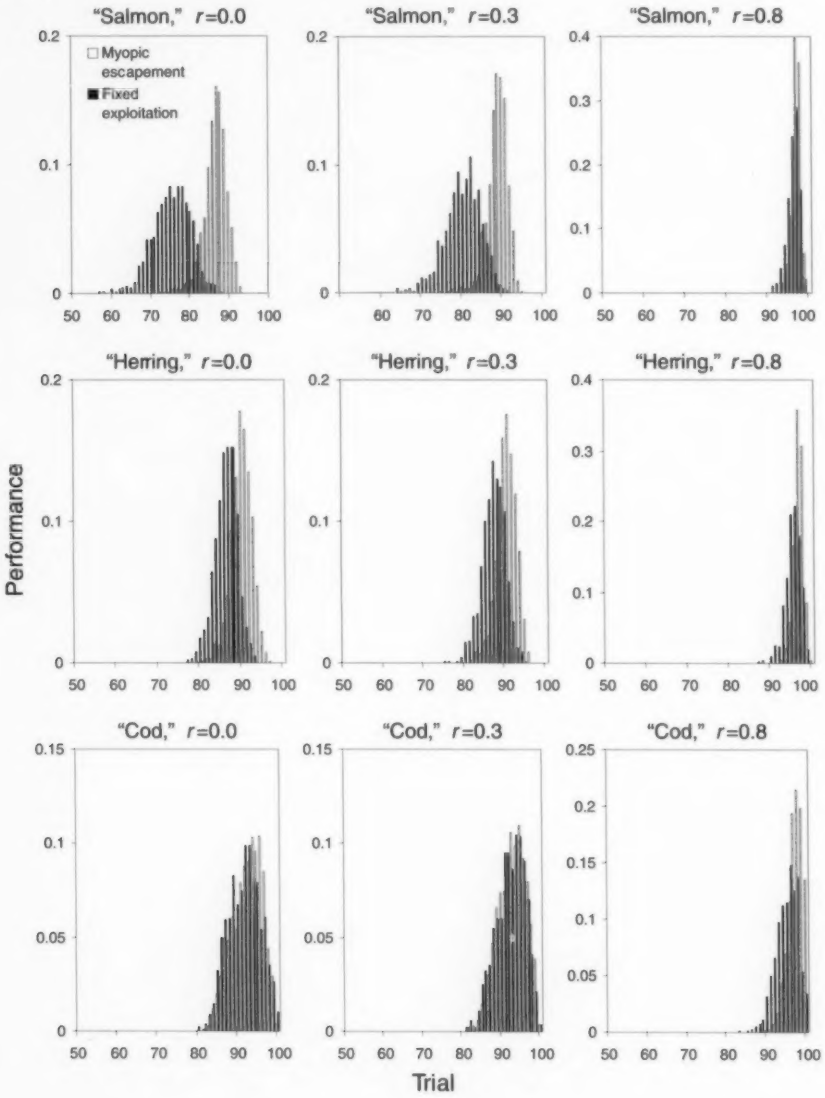
The results in Fig. 5 can be misleading for fisheries where the only practical way to implement a harvest strategy is to set (and live with) each annual allowed catch (TAC) by using a preseason biomass estimate, at either $\text{TAC} = \hat{B} - S_t$ or $\text{TAC} = u_e\hat{B}_t$, if the biomass estimate has high random error. Walters (1989) showed substantial degradation in the performance of optimal escapement policies with decreasing accuracy of preseason forecasts, even though these optimal policies were actually derived by acknowledging the uncertainty in the biomass estimates. Stock assessment methods like production model fitting or VPA tuning to relative abundance time series typically result in $\hat{B}_t$ with substantial error that is approximately log-normally distributed and, for long-lived species, strongly autocorrelated (e.g., Anonymous 1991; Parma 1993). That is,

$$[8] \qquad \hat{B}_t = B_t e^{b_t}$$

$$b_t = kb_{t-1} + v_t$$

where v_t represents independent annual errors resulting from both observation and biological process uncertainty. The autocorrelation factor k can be as high as g_e, which can be shown using a Kalman filter to describe or approximate the estimation procedure (see Pella 1993 and Schnute 1994 for description of the Kalman filter model). Note how g_e acting as an autocorrelation factor tends to produce larger swings in the biomass estimates over time than would be predicted from annual variation in relative abundance or tuning survey data, with runs of years when the estimates are too high or low. But whether or not the assessment errors are autocorrelated, simulated management performance degrades rapidly with increases in the standard deviation of b_t (approximately the coefficient of variation of the error of the biomass estimates) as shown in Fig. 6. To obtain each plotted point in Fig. 6, we took subsets of 10 of the environmental pattern scenarios used for Fig. 5, and ran ten 100-year simulations for each scenario with catch set each year to TAC calculated from randomly varying $\hat{B}_t$ and autocorrelated error with $k = g_e$. Initial biomass in each case was set to the equilibrium level corresponding to u_e and average environmental conditions. Results for $g_e = 0$ (salmon case) confirm the finding of Eggers (1993) that the catch performance of fixed exploitation policies degrades more rapidly with increasing assessment error (coefficient of variation of $\hat{B}_t$) than does the performance of spawning stock S^*, S' strategies. This effect is not as marked for intermediate life history parameters (herring case), where the fixed exploitation policy performs similarly to the myopic one, except for very high estimation errors (CV $\geq$ 0.75). The average yield obtained under the three policies at CV values above 0.75 drops to less than 40–50% of that obtained when CV = 0. Interestingly, u_e policies continue to perform relatively well for long-lived, unproductive stocks (where u_e is very low in

Fig. 5. Distributions of performance for fixed exploitation and myopic escapement strategies, measured relative to theoretical optimum if all future environmental changes were known; each distribution represents 1000 100-year simulation trials where each trial would look qualitatively like the results in Fig. 4. Alternative life-history types are described in the text ("salmon" is semelparous, productive; "herrring" is iteroparous but short lived; "cod" is long lived and unproductive).

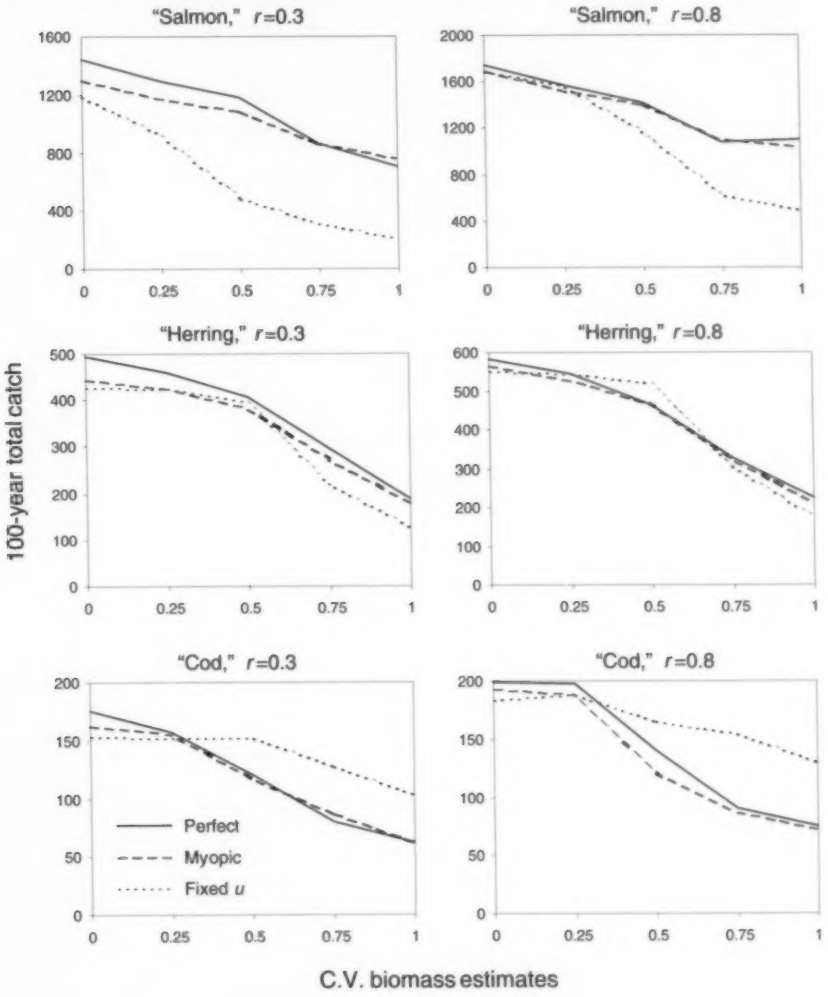


the first place) even when the simulated biomass assessments are very highly autocorrelated and noisy (commonly swinging over many years to as low as 20% of actual B, or as high as 300% of B, in the CV = 1.0 cod case of Fig. 6). Escapement strategies (S^* and S'), on the other hand, perform much worse than u_e policies, which suggests that adjusting escapement targets to changing environmental conditions may even be counterproductive when assessment errors are large and highly autocorrelated. Overall, these results suggest that careful attention must be paid to the question of how to implement harvest strategies, and this we discuss in the next section.

Implementation of exploitation rate policies

Two fundamental requirements for practical implementation of an optimal exploitation rate policy are that (*i*) the average slope of the recruitment relationship at low stock sizes (A of

Fig. 6. Effect of stochastic errors in annual biomass estimation on performance of escapement and fixed harvest rate strategies. Results show how performance for simulations as in Figs. 4 and 5 degrades with increase in coefficient of variation in annual biomass estimates. Biomass estimation procedures were assumed to result in autocorrelated estimation errors with the same persistence time as the effects of biological recruitment variations (as measured by the growth–survival factor g). Degradation in performance for fixed exploitation strategies was calculated by assuming worst-case implementation where annual catch (TAC) is set as exploitation rate times biomass estimate (no direct regulation of probability of capture). The severity of this degradation is related mostly to the target exploitation rate (50% for "salmon," 45% for "herring," 10% for "cod"); large, persistent estimation errors were actually most severe in the "cod" simulation cases where degradation is least severe.



eq. 2) must be known, along with the growth–survival parameters that determine g_e, and (*ii*) a regulatory apparatus must monitor and limit the annual exploitation rate. The implementation of myopic escapement policies (S') further requires knowledge of the recruitment carrying capacity parameter K each year (or M_{it}), and an accurate estimate of the current stock biomass B_t (or a means to estimate B_t or S_t adaptively within the fishing season). Thus the implementation requirements for escapement policies are in principle substantially greater than for exploitation rate policies. Although implementation of fixed exploitation policies also would seem to require accurate estimates of both catch and B_t (e.g., Eggers 1993), this information is needed only in cases where either (*i*) a preseason allowable catch (TAC) is based on a biomass estimate (TAC = $u\hat{B}$ as in the simulation results of Fig. 6), and catch is limited by tactics such as individual vessel quotas or closure

when TAC is reached, or (*ii*) exploitation is permitted in several adaptive steps within each year, but over fishing areas or seasons of such size and duration as to make the exploitation rate sensitive to fishing effort characteristics (catchability, fleet size) during the fishery openings. A particular concern in such cases is the depensatory effect of overestimating stock size when it is low (u consistently too high when B_t is low: see for examples Eggers 1993 and Parma 1993). The next section addresses two key questions: (*i*) Can we assess A or u_e without destructive overfishing along the way? (*ii*) Are there harvest regulation tactics that can prevent the potential depensatory effects of overestimating TAC or underestimating fishing efficiency at times when B_t is low, and in particular are there tactics that do not depend at all on knowledge of total stock size?

In principle, certainty about the recruitment curve slope A at low spawning stock sizes is only possible by observing a substantial number of recruitments under such spawning stock conditions. To suggest that we can predict A from an understanding of its biological components, f and M_1, is plainly naive. But the conditions when A becomes "visible" (recruitment proportional to stock, hence failing as stock declines) are the defining circumstances for recruitment overfishing. Opportunities now abound to gather such observations around the world, as fisheries collapse with monotonous regularity. Short of such hard experience, comparative approaches seek to predict A for a given stock from information about similar stocks elsewhere. Some useful patterns emerge from two such approaches: (*i*) study of historical stock-recruit data for at least identifying spawning stock size below which recruitment begins to fall rapidly (Sissenwine and Sheperd 1987; Mace 1994; Myers et al. 1994), and (*ii*) study of simpler stock trend data (Patterson 1992) to provide direct empirical evidence about the sustainable u_e. Patterson's (1992) finding that pelagic stocks have generally been able to sustain exploitation rates of approximately only 0.5 to 1 times the natural mortality rate, as predicted from some modeling studies (reviewed in Patterson 1992), appears to work for demersal species as well. A worrisome point about the Patterson (1992) finding is that the popular $F_{0.1}$ harvest rate, which usually implies $F \approx M$ and is generally considered to be quite conservative (Deriso 1987), may in fact be too high for the majority of natural populations. Because underestimation of the optimal exploitation rate for long-lived species is not particularly costly (see discussion above on derivation of u_e: using a value of u that is too low results in a compensating increase in stock biomass), we consider the prudent approach to assume $u_e \leq 0.5\ M$ and to place the burden of proof on whoever advocates a higher rate to demonstrate that it is sustainable (by substantial direct analysis of historical stock-recruit data). Therefore, our answer to the first question above is yes; we can assess u_e without destructive overfishing, provided fisheries managers are willing to operate with a standard of prudence that recognizes the modest cost of underestimating the optimum to be much less than the major costs and social upheaval often attendant upon overestimating it.

Modern fishing technology makes impractical the use of effort regulation alone to achieve stable exploitation rates, via some simple relation like $F = qE$ with catchability q constant (Cooke and Beddington 1985). Generally q will increase dramatically with decreases in stock size and with further technology development over time. Hyperdepletion situations (Hilborn and Walters 1992), where q actually decreases with stock size, appear to be rare and to be associated mainly with recreational fisheries and with some sedentary species where aggregations are slow to reform once cropped. However, these problems with q do not imply that space–time restriction of fishing effort will fail in general; they apply only to situations where a large proportion of the stock is exposed to fishing at once. In some Pacific salmon fisheries, like seine fisheries for Fraser River sockeye (*Oncorhynchus nerka*) during their extended spawning migration through restricted coastal straits, exploitation rates have been quite successfully restricted by allowing only very short fishery openings in small areas. In these situations, virtually every fish present in or entering the open areas is likely taken during each opening (so further technology improvement will just mean taking them sooner within each opening); such fisheries literally chop holes in the spatial distribution of the stocks as they migrate past fishing areas, and u is simply the proportion of fish exposed to fishing, i.e., the proportion of fish that do not pass through the fishing areas during closures. Placing massive amounts of effort in competition within very small fishing areas over short times may be uneconomical, but it guarantees that u remains below some target u_e established through long-term population analysis. Similar spatial closure strategies could be developed for nonmigratory species as well, for example, by having small open areas that are rotated over the stock distribution from year to year (e.g., Botsford et al. 1993; Pfister and Bradbury 1996). The potential of year-around closed areas as a management tool is now recognized and issues related to the design and evaluation of harvest refugia are now beginning to be discussed (e.g., Polacheck 1990; Roberts and Polunin 1991; Dugan and Davis 1993; Rowley 1994). Therefore, the answer to our second question above is again yes, provided area–time restrictions that create uneconomical competition (and may be very expensive to enforce) are considered an acceptable cost of insuring that exploitation rates are never too high, and that we are willing to gather the detailed spatial distribution and movement data needed to set areas and times correctly.

Area–time effort restrictions are not the only way to directly regulate exploitation rates without accurate estimates of catch and stock size. Conduct of a careful annual preseason tagging program, with monitoring of the tag recovery rate, may provide a direct measure of exploitation rate as fishing progresses. However, this approach would not work if substantial tag loss, mortality, or underreporting of tags occurs, as these problems can lead to dangerous underestimates of the exploitation rate (fewer fish recovered than should be on the basis of the actual probability of harvest). Another option is to accompany fishing activities by large-scale abundance indexing (e.g., acoustics, survey fishing) tied directly to the spatial distribution of fishing so as to quickly measure relative depletion rates (exploitation rates) within the fishing areas. Still another (and perhaps last) resort is to revive the very old tactic of restricting fishing gears (e.g., hook and mesh sizes) so that only a safe proportion of the population size distribution is landed or retained. None of these ideas is new at all; they have not received much attention in recent years, perhaps because we have placed considerable faith in our ability to improve stock size and catch assessments enough to make many regulatory tactics unnecessary.

With strong public demand for more cautious approaches to sustainable fishery management, fisheries management agencies (and fishing industry cooperators) will very likely be forced in the future to combine increased investment in improved assessments of absolute stock size with fail-safe regulatory measures including large, closed areas, short seasons, and selective harvest of larger fish. Such combined approaches will create long-term incentives for collaboration between the scientific and industrial communities in gathering better data for assessment, because it should be possible to progressively relax the fail-safe measures that create uneconomical fishing situations if more reliable assessments do become available. It is not yet clear just how far this trade-off can be taken, especially considering that much of the recent fisheries stock assessment literature has focused on showing how large our assessment errors are rather than showing how these errors can be substantially reduced.

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An extended cohort analysis: incorporating the effect of seasonal catches

G. Mertz and R.A. Myers

Abstract: A virtual population analysis relates changes in numbers at age of fish to commercial catch, fishing mortality, and natural mortality. We reformulate the standard virtual population analysis and corresponding catch equation in a manner that incorporates any seasonal pattern of catches, which may represent continuous fisheries or pulse fisheries. An advantage of this treatment is that it leads to a virtual population analysis formulated as cohort analysis, with $e^{M/2}$ replaced with a factor representing (and easily calculable from) the particular seasonal pattern of catches. The method presented here circumvents the standard assumption (leading to the Baranov catch equation) that fishing mortality is constant throughout the year.

Résumé : Une analyse de la population virtuelle établit un rapport entre les changements dans l'effectif selon l'âge du poisson et les prises commerciales, la mortalité par pêche et la mortalité naturelle. Nous reformulons l'analyse de la population virtuelle standard et l'équation de capture correspondante d'une façon qui intègre les profils saisonniers des captures, qui peuvent représenter des pêches en continu ou à caractère pulsatoire. L'avantage de cette méthode est qu'elle donne une analyse de la population virtuelle formulée comme une analyse de cohorte, $e^{M/2}$ étant remplacé par un facteur représentant (après un calcul facile) le profil saisonnier particulier des captures. La méthode présentée ici contourne l'hypothèse standard (qui mène à l'équation de capture de Baranov) selon laquelle la mortalité par pêche serait constante tout au long de l'année.
[Traduit par la Rédaction]

Introduction

Most of the world's major fisheries are managed using statistical catch-at-age models that relate commercial catch-at-age to indices of abundance such as research surveys or commercial catch per unit effort indices (Hilborn and Walters 1992). These models describe the relationship between population abundance, fishing mortality, natural mortality, and the commercial catch. The most common assumption made is that fishing mortality is constant throughout the year, which leads to the Baranov catch equation. The Baranov catch equation implies a nonlinear equation for virtual population analysis (VPA); that is, the numbers of fish in a cohort in year i are not linearly related to either the catch or the numbers in year $i + 1$. The VPA equation may be solved iteratively or approximated using a variety of approaches (Pope 1972; Sims 1982*a*; MacCall 1986; Allen and Hearn 1989; Evans 1989). Of these approaches, the one that is almost always used in practice is Pope's approximation (described below), which allows a linear VPA. Pope's formulation is exact for a pulse fishery occurring at midyear, although it is often regarded as an approximation to the Baranov-based approach (Hilborn and Walters 1992).

The methods listed above generally assume that the Baranov catch equation is exact (or occasionally that Pope's formula is exact). In fact few fisheries will conform to either the Baranov pattern of constant fishing mortality throughout the year (MacCall 1986; Evans 1994) or the Pope pattern (a pulse fishery at midyear). Here, we present a method that explicitly allows seasonal variations in catch rates (catch per unit time) and that may represent continuous fisheries or pulse fisheries. This technique provides the benefit of a VPA equation that takes the form of Pope's (1972) cohort analysis, requiring only the replacement of $e^{M/2}$ with a factor representing (and that is easily calculable from) the seasonal pattern of catches for the stock in question. Our approach further allows the derivation of a generalized catch equation, which is not limited to a Baranov type fishery. Our results are easily implemented in any of the standard approaches to the analysis of catch-at-age data.

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Analysis

We will proceed from the following equation, governing the evolution of numbers in a cohort (N) through time:

$$[1] \quad \frac{dN}{dt} = -mN - C'$$

where m is the instantaneous natural mortality rate (having units of time to the minus one) and $C' \equiv dC/dt$ (C represents

catch) is the number of fish being caught, at an instant, per unit time; that is, dC/dt is the instantaneous catch rate. The instantaneous natural mortality rate is related to the annual mortality rate M through $M = m \times (1 \text{ year})$ when M is constant.

Assuming only that m is constant, a general solution to eq. 1 (see, e.g., Ritger and Rose 1968, section 2-3) can be obtained:

$$[2] \quad N(t_1) - e^{-mt_1} N(0) = -e^{-mt_1} \int_0^{t_1} e^{mt} C' dt$$

where $N(0)$ is the initial number in the cohort and $N(t_1)$ is the number at $t = t_1$.

As catch data are generally available on a per annum basis, we will now recast eq. 2 to reflect our interest in annual changes. Now, $t = 0$ in eq. 2 will be the beginning of a given year, indexed by i, and $t = t_1$ will be the end of the year. Thus, $N(0)$ becomes N_i and $N(t_1)$ becomes N_{i+1}. Also, since $t_1 =$ 1 year, we have $mt_1 = M$. In all forthcoming equations, t will refer to time within the span of a year and will be expressed as a fraction in the range 0–1. Equation 2 may now be written as

$$[3] \quad N_{i+1} - e^{-M} N_i = -e^{-M} \int_0^1 e^{Mt} C_i'(t) dt$$

We have also made the catch rate year specific. Equation 3 includes the limit in which the catch is taken in a series of pulse type fisheries, as envisioned by Sims (1982*b*) and MacCall (1986). Sims (1982*b*) applied Pope's formula to each of a number of subintervals of a year, and thus his eq. 3 will be exact if the fishery consists of series of pulse catches at the middle of each subinterval. Similarly, MacCall (1986), in his eq. 15, derives a population equation that is exact for a series of pulse catches at known times.

By writing $\tilde{C}_i = e^{-M} \int_0^1 e^{Mt} C_i'(t) dt$, we can express eq. 3 as

$$[4] \quad N_{i+1} - e^{-M} N_i = -\tilde{C}_i$$

where $\tilde{C}_i$ could be termed the adjusted catch, in that it represents a weighted integral over time of the catch rate. If catch rate data are available, perhaps on a monthly basis, for the fishery of interest, the integral in eq. 3 may be replaced with the appropriate sum over months. Equation 4 provides for a simple VPA; once the $\tilde{C}_i$ values are calculated, N_i is easily computed from N_{i+1}. In fact the VPA formulated has the advantage that no fishing mortality term appears, which would necessitate (as in the standard VPA) a new approximation to linearize the equation or an iterative solution. Equation 4 as it stands is complete, and the analysis could be terminated here. Although eq. 4 is sufficient for a VPA analysis, we have not dealt with the issue of how significantly the adjusted catch $\tilde{C}_i$ departs from the actual catch C_i that is given by $\int_0^1 C_i' dt$. This will be taken up immediately.

To compare C_i and $\tilde{C}_i$ we introduce the ratio

$$[5] \quad \gamma_i(M) = \frac{\tilde{C}_i}{C_i} = \frac{e^{-M} \int_0^1 e^{Mt} C_i'(t) dt}{C_i}$$

This ratio gauges the extent to which the seasonal pattern in catch rate affects the population analysis. We will be particularly interested in cases for which $\gamma_i(M)$ can be treated as a constant (negligible interannual variations). In the material to be presented we will write $\gamma_i(M)$ as $\gamma(M)$ in cases where the interannual variability can be ignored.

A constant $\gamma_i(M)$ will result if the seasonal catch rate pattern does not change from year to year. The factor $\gamma_i(M)$ will be constant if $C_i'(t)$ can be written as $C_i'(t) = A_i c'(t)$, a year effect, A_i, multiplied by a catch rate profile, $c'(t)$, where $c'(t)$ describes the changes in catch rate within a year. For example, we could specify $c'(t) = \sin(\pi t)$, a function that describes a fishery peaking in midyear.

We can now write eq. 3 as

$$[6] \quad N_{i+1} - e^{-M} N_i = -\gamma_i(M) C_i$$

Below we will establish bounds for $\gamma_i(M)$ and we will describe situations under which $\gamma_i(M)$ may be treated as a constant.

Extended cohort analysis

Equation 6 can be rewritten as

$$[7] \quad N_i = e^M N_{i+1} + \gamma_i(M) e^M C_i$$

which may be recognized as a close analog of cohort analysis (Pope 1972). In fact, by specifying a pulse fishery at midyear, eq. 5 immediately yields $\gamma_i(M) = \gamma(M) = e^{-M/2}$ and, after substitution into eq. 7, one recovers Pope's (1972) result. The so-called discrete fishery (Hilborn and Walters 1992; section 10.2) consists of a pulse fishery at the end of the year; one can immediately calculate $\gamma(M) = 1$, which yields the correct VPA equation when substituted into eq. 7. In general, a pulse fishery at time α ($0 \le \alpha \le 1$) gives $\gamma(M) = e^{-M(1-\alpha)}$ with the corresponding VPA equation being

$$[8] \quad N_i = e^M N_{i+1} + e^{-M\alpha} C_i$$

which is the natural generalization of cohort analysis for pulse fisheries.

These results for the pulse fisheries have a straightforward interpretation: in eq. 6 we fully discount N_i by the factor e^{-M}; however, if the catch is removed immediately at the beginning of the year, we actually should discount the numbers given by $N_i - C_i$, and hence the appearance of the term $e^{-M} C_i$ in eq. 6, for this case. If the catch is taken at the end of the year, then full discounting of N_i is appropriate and no product of natural mortality and catch term arises. In view of this, we might term $\gamma(M)$ the mortality correction factor.

The limits for $\gamma_i(M)$ are defined by the two extreme cases of a pulse fishery at the beginning of the year and a pulse fishery at the end of the year. For a pulse fishery at the end of the year, no correction is necessary for the application of natural mortality and $\gamma_i(M) = 1$. Pulse fishing at the beginning of the year implies that we have overdiscounted N_i by the maximum amount possible, specifically, $C_i e^{-M}$ (in other words, $\gamma_i(M) = e^{-M}$). Thus, with complete generality we can state

$$[9] \quad e^{-M} \le \gamma_i(M) \le 1$$

These considerations explain the success of Pope's (1972) cohort analysis: by in effect adopting the compromise value of $\gamma_i(M) = \gamma(M) = e^{-M/2}$, Pope's treatment can never be in error by more than $e^{-M/2} - e^{-M}$ or $1 - e^{-M/2}$. If M is not too large then both these expressions are approximately equal to $M/2$, so that for a typical M of 0.2 the error can never be worse than about 10%.

Treating γ as a constant

In this subsection we address the issue of when $\gamma_i(M)$ can be treated as a constant, i.e., when its interannual variability can be ignored. The direct procedure is to calculate $\gamma_i(M)$ numerically for each year, say from monthly catch rate data (catch per month):

$$[10] \quad \gamma_i(M) = \frac{e^{-M}\sum_{n=1}^{n=12} e^{((n-1/2)/12)M} C_i'(n)}{\sum_{n=1}^{n=12} C_i'(n)}$$

where we have assumed that the catch rate data represent the center of each month, indexed by n (n = 1 to 12). Of course, more sophisticated numerical approximations to the integral in eq. 5, such as Simpson's rule, can be employed, if desirable. The calculated values of $\gamma_i(M)$ can then be inspected to determine if the interannual variations are small enough to be neglected. However, some simple analysis, presented below, shows that numerical treatment is unnecessary in many cases.

For an analytic treatment, consider first the example of a seasonally limited fishery with reasonably constant catch rates. This might occur when a fisheries operations manager matches catches to plant capacity, say maintaining the catch rate at a specified number of tons per week. Consider the case where the fishery is centered on midyear, for comparison to cohort analysis; specifically, the catch is taken at a constant rate for a period from 0.5 – α/2 to 0.5 + α/2. We find

$$[11] \quad \gamma(M) = e^{-M/2}\frac{e^{M\alpha/2} - e^{-M\alpha/2}}{M\alpha}$$

Clearly as α shrinks to zero the cohort analysis result of $\gamma(M) = e^{-M/2}$ is recovered. However, even in the extreme case of α = 1, agreement with the cohort analysis result is very good. From eq. 11, γ(0.2) = 0.906 and γ(0.5) = 0.787, while from the cohort analysis, γ(0.2) = 0.905 and γ(0.5) = 0.779. For a sinusoidal catch rate profile, $c'(t) = \sin(\pi t)$, $\gamma(M) = (1/2)(1 + e^{-M})((M/\pi)^2 + 1)^{-1}$, we have γ(0.2) = 0.906 and γ(0.5) = 0.783. It is evident that cohort analysis, stipulating a pulse fishery at midyear, is an excellent approximation to the two continuous fisheries treated, constant catch throughout the year and the sinusoidal catch rate profile.This is a consequence of the fact that these latter two fisheries are symmetric about their centroids, so that $\gamma(M)$ is extremely insensitive to the shape of the catch rate profile, and its year-to-year constancy is assured. California mackerel (Fig. 2 of MacCall 1986) provides a possible example in that the monthly catches are approximately symmetrical about midyear.

There will be cases where the catch rate profile varies appreciably from year to year; for example, fishing mortality could be constant within a year (a Baranov type fishery) but vary among years, in which case the shape of the catch rate curve changes interannually. In many such examples it will be possible to exploit seasonal limitations on the fishery to justifiably invoke a constant $\gamma(M)$. If the fishery is confined to the interval α_1 to α_2 ($\alpha_2 \geq \alpha_1$, $0 \leq \alpha_1$, $\alpha_2 \leq 1$) then, using the same arguments used to justify eq. 9, we can write

$$[12] \quad e^{-M\alpha_2} \leq \gamma_i(M) \leq e^{-M\alpha_1}$$

which is, of course, a direct analog of eq. 9. The natural choice for $\gamma_i(M)$ is simply, in the spirit of Pope's cohort analysis, $\gamma_i(M) = \gamma(M) = e^{-M(\alpha_1 + \alpha_2)/2}$. If the arguments of the exponentials are not large, the maximum possible error resulting from this choice of $\gamma_i(M)$ will be approximately $M(\alpha_2 - \alpha_1)/2$. For a 3-month fishery ($\alpha_2 - \alpha_1 = 0.25$), with $M = 0.2$ the maximum possible error is about 2.5%. Even for $M = 0.5$ the maximum possible error is about 6%. Bear in mind that these errors are extremes that would only be encountered if the fishery fluctuated between a pulse fishery at the beginning of the 3-month interval and one at the end of the 3-month interval. Thus, seasonal limitation of the fishery will often justify treatment of $\gamma(M)$ as being constant from year to year (despite changes in the catch rate profile).

A related problem will arise if it is suspected that the centroids of the catch versus time (within a year) curves change with age group. The significance of the shift can be assessed by applying an error analysis similar to that given in the previous paragraph. One can easily show that $\Delta\gamma_i(M)/\gamma_i(M) \approx M\Delta\alpha$, where $\Delta\alpha$ is the centroid shift and $\Delta\gamma_i(M)$ is the corresponding change in $\gamma_i(M)$. Thus, a 3-month difference in the centroids of the catches of two age groups ($\Delta\alpha = 0.25$) would result in a 2.5% change in $\gamma_i(M)$ (for $M = 0.2$).

Finally, we have applied eq. 10 to two real fisheries, as described in the next two paragraphs.

We calculated $\gamma_i(M)$ for northern cod, i.e., cod off Labrador and the northern Grand Banks. This resource has traditionally been prosecuted primarily during the summer and was one of the stocks for which Pope developed his approximation. We have calculated $\gamma_i(M)$ (Fig. 1), for $M = 0.2$ (the accepted assessment value), for each year from 1954 to 1991 using data compiled and described by Hutchings and Myers (1995). The seasonal patterns for the catches for the years of maximum and minimum $\gamma_i(M)$ are shown in Fig. 1B. Pope's approximation (broken line) is usually correct within 3% for this fishery. The trend in the $\gamma_i(M)$ over time is caused by interannual shifts in the timing of the offshore fishery. In the early 1950s there was virtually no spring fishery because of sea ice. Trawlers that could operate in ice were introduced in the late 1950s and the largest catches were taken in the spring on prespawning concentrations (Hutchings and Myers 1995). Despite the changes in seasonality, the variations in $\gamma_i(M)$ are generally weak (for reference note that northern cod catches changed by more than a factor of four in the plotted period). Although the northern cod fishery is far from being a pulse fishery, it is evident that the Pope (1972) formulation excellently approximates the true $\gamma_i(M)$.

In Fig. 2 we present the $\gamma_i(M = 0.2)$ series, and seasonal catch patterns for the years of maximum and minimum γ_i, for herring of the east and south coasts of Newfoundland. Herring catches are generally bimodal with peaks in the spring and fall, and there is a secular shift evident, from a dominant peak in spring, for the early segment of the record, to a dominant peak in fall, for the latter portion of the record. In the late 1980s the discrepancy between the true $\gamma_i(M)$ and Pope's approximation becomes greater than 5%, but it is still notable that Pope's approximation remains quite reliable even for this fishery that does not conform to the Pope pattern of peaking at midyear.

To summarize the material presented thus far, in this section, we note that our examination of the behavior of $\gamma_i(M)$ has shown that its interannual variability will in many

Fig. 1. (A) The factor $\gamma_i(M = 0.2)$ calculated from eq. 10 for northern cod, i.e., cod off Labrador and the northern Grand Banks. The broken line is Pope's approximation. (B) Monthly catches expressed as percentages of the total for the years of maximum and minimum $\gamma_i(M)$.

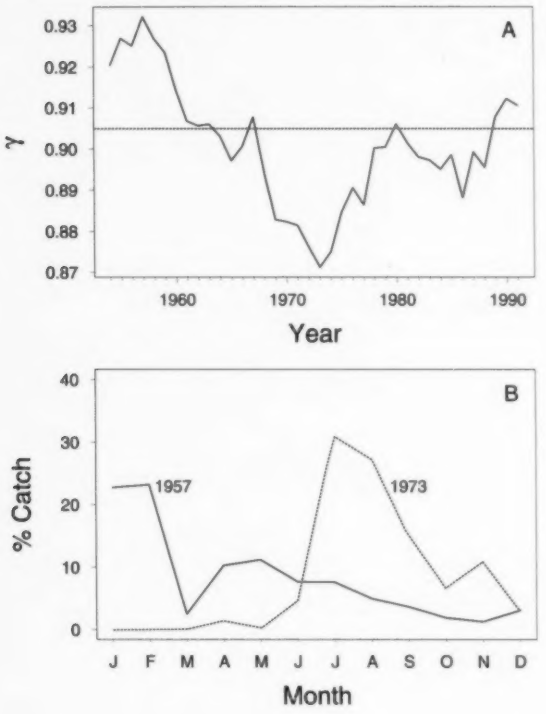
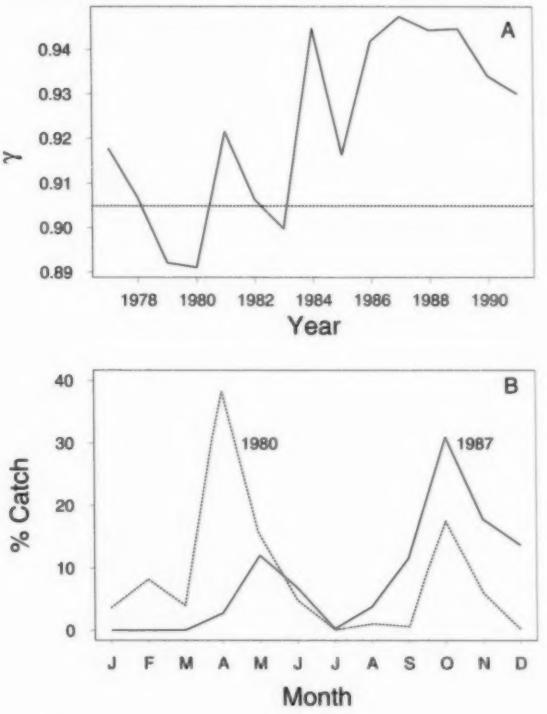


Fig. 2. (A) The factor $\gamma_i(M = 0.2)$ calculated from eq. 10 for south and east coast Newfoundland herring. The broken line is Pope's approximation. (B) Monthly catches expressed as percentages of the total for the years of maximum and minimum $\gamma_i(M)$.



circumstances be much less than that of catches, suggesting that catches can be entered in the population model with a constant $\gamma(M)$. (Equivalently, the interannual variability in the adjusted catch, $\tilde{C}_i$, will be almost entirely due to changes in catch.) By formulating our population model, eq. 6 or eq. 7, with the factor $\gamma_i(M)$ we have emphasized the continuity and compatibility with Pope's (1972) cohort analysis.

The catch equation

A general catch equation, not specific to any particular seasonal fishing pattern, may be obtained from eq. 6. We require the following equation:

$$[13] \quad N_{i+1} = N_i e^{-(F_i+M)}$$

where F_i is the fishing mortality (which is defined by this equation). Equations 6 and 13 may be used to show that

$$[14] \quad \gamma_i(M)C_i = \tilde{C}_i = N_i(e^{-M} - e^{-(F_i+M)}) = (1 - e^{-(F_i+M)})N_i - (1 - e^{-M})N_i$$

Equation 14 may be interpreted as follows: the adjusted catch is equal to the numbers perishing from fishing plus natural mortality less the numbers that would perish from natural mortality if the entire catch were taken at the end of the year. Note that the catch is bounded by the limits $e^{-M} N_i(1 - e^{-F_i})$ and $N_i(1 - e^{-F_i})$, a result derived earlier by Mesnil (1980).

The standard, or Baranov, catch equation is given by

$$C_i = \frac{F_i}{F_i + M}(1 - e^{-(F_i+M)})N_i$$

and it may be shown to be a special case of eq. 14. If we specify constant fishing mortality throughout the year, $C_i(t) = A_i \exp(-(F_i + M)t)$, then substitution of this form of $C_i(t)$ into eq. 5 yields

$$[16] \quad \gamma_i(M) = \frac{F_i + M}{F_i} \frac{e^{-M} - e^{-(F_i+M)}}{1 - e^{-(F_i+M)}}$$

and substitution of this $\gamma_i(M)$ into eq. 14 gives the Baranov catch equation, as it must.

Discussion

Many previous studies have taken the Baranov catch equation to be exact, and considerable effort has been expended to preserve this exactness in the VPA, by finding accurate, rapidly converging iterative solutions or accurate linear approximations to the nonlinear VPA resulting from the Baranov catch equation. Once it is acknowledged that the Baranov catch equation is, in general, an approximation (because catch rates may be very seasonal), considerable simplification is possible. The approach encapsulated in eq. 6 and eq. 7 permits

the incorporation of realistic catch rate profiles while also providing the benefit of a VPA that is as simple as, and closely analogous to, cohort analysis. The significance of interannual variations in $\gamma_i(M)$ can be investigated through application of the bounds in eq. 12, or through direct calculation (using eq. 10) when detailed catch information is available. By applying eq. 10 to the northern cod fishery we were able to demonstrate that Pope's approximation is accurate to within about 3% for this example. In cases where the interannual variability of $\gamma_i(M)$ is not negligible, one still acquires, with our approach, the advantage of a VPA resembling cohort analysis (N_i is immediately calculable from N_{i+1} and C_i) once the $\gamma_i(M)$ series is calculated.

We should emphasize that the accuracy of Pope's, or any other, approximation for a particular fishery should be established by estimating the $\gamma_i(M)$ rather than by comparing the approximation with the Baranov result. In fact, we would recommend calculating the $\gamma_i(M)$ series whenever sufficient catch data exist. If monthly catch data are not available, $\gamma_i(M)$ can still be estimated from approximate knowledge of the timing of the fishery (see discussion near eq. 12) and the adequacy of the estimate of $\gamma_i(M)$ can be evaluated using eq. 12.

A VPA formulation taking the form of a generalized cohort analysis, such as eq. 7, is a considerable advantage to methods that employ Kalman filters and state space models, which have relied upon the linearity to incorporate estimation error in the catch-at-age (Collie and Sissenwine 1983; Mendelssohn 1988; Gudmundsson 1994). Linearity may be achieved by assuming that the fish are caught in a pulse at the beginning of the year (Collie and Sissenwine 1983; Mendelssohn 1988; Walters and Punt 1994). This is equivalent to assuming that $\gamma_i(M) = e^{-M}$. Our method can be used to provide the $\gamma_i(M)$ appropriate to the actual catch profile, and in many cases will justify its treatment as a constant. The same linearity means that analytical derivatives can be easily estimated in models with complex error structure (Walters and Punt 1994; Myers and Cadigan 1995) or more complex models that include size structure (Schnute et al. 1989). In the catch-at-age model most often used by the International Council for the Exploration of the Sea, called extended survivors (J.G. Shepherd, MAFF Fisheries Laboratory, Suffolk NR33 OHT, England, unpublished data), Pope's (1972) approximation is key to the estimation algorithm. All of these methods can be easily generalized by using our results.

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Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes

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Abstract: Epilithic organic matter, all aquatic macroinvertebrates except shredders, and fish were significantly enriched with ^{15}N and ^{13}C in streams (western Washington state, U.S.A.) where spawning coho salmon (*Oncorhynchus kisutch*) were present. Riparian vegetation adjacent to salmon-bearing streams and shredding macroinvertebrates were enriched with ^{15}N but not ^{13}C. The highest levels of enrichment of the stream biota with the heavier isotopes occurred in the early spring, shortly after carcasses had decomposed. Following spawning, age-0 coho salmon exhibited a doubling in rate of growth. Age-0 cutthroat trout in a nearby stream without salmon exhibited no change in growth rate during the winter. Salmon-derived organic matter was incorporated into the stream biota through direct consumption of eggs, carcasses, and fry and by sorption onto the streambed substrate of dissolved organic matter released by decomposing carcasses. Autotrophic uptake was not an important avenue of incorporation. The proportion of nitrogen contributed by spawning salmon varied among trophic categories, ranging from about 17% in collector-gatherers to more than 30% in juvenile coho salmon. Carbon contributed by spawning salmon ranged from 0% in the foliage of riparian plants and shredders to 34% in juvenile coho salmon.

Résumé : On observe un enrichissement notable en ^{15}N et ^{13}C de la matière organique épilithique, de tous les macroinvertébrés aquatiques sauf les broyeurs, et des poissons, dans des cours d'eau (ouest de l'état de Washington, États-Unis) où sont présents des géniteurs de saumon coho (*Oncorhynchus kisutch*). La végétation riveraine des cours d'eau salmonicoles et les macroinvertébrés broyeurs étaient enrichis en ^{15}N mais pas en ^{13}C. Les niveaux les plus hauts d'enrichissement du biote des cours d'eau en isotopes lourds ont été notés au début du printemps, peu de temps après la décomposition des carcasses. Après la ponte, les saumons d'âge 0+ ont connu un doublement du taux de croissance. Les truites fardées d'âge 0+ dans un cours d'eau proche exempt de saumons n'ont présenté aucun changement du taux de croissance pendant l'hiver. L'incorporation de la matière organique provenant des saumons dans le biote du cours d'eau se faisait par consommation directe des oeufs, des carcasses et des alevins, et par sorption dans le substrat du fond du cours d'eau de la matière organique dissoute libérée par les carcasses en décomposition. L'assimilation autotrophe n'est pas un moyen important d'intégration. La proportion d'azote apportée par les saumons géniteurs variait selon les catégories trophiques, d'environ 17% chez les cueilleurs-capteurs à plus de 30% chez les cohos juvéniles. Le carbone apporté par les saumons allait de 0% dans le feuillage des plantes ripicoles et chez les broyeurs à 34% chez les cohos juvéniles.
[Traduit par la Rédaction]

Introduction

Many species of Pacific salmon (*Oncorhynchus* spp.) of northwestern North America are anadromous and semelparous (i.e., they die after spawning once). Nutrients accumulated in biomass by these fish while in the ocean are transported back to their freshwater spawning sites. Pink salmon (*Oncorhynchus gorbuscha*) and sockeye salmon (*Oncorhynchus nerka*), species that typically spawn at high densities, have been shown to make a significant contribution of nutrients to the freshwater ecosystems they inhabit. Pink salmon returning to Sashin Creek in southeastern Alaska produced a month-long, fourfold increase in NH_4^+ concentration and threefold increase in dissolved organic nitrogen concentration (Brickell and Goering 1970). Richey et al. (1975) associated spawning of kokanee salmon (landlocked *O. nerka*) with elevated nitrogen and phosphorus concentrations and an increase in autotrophic production and heterotrophic activity in a tributary of Lake Tahoe, California. Sockeye salmon carcasses are an important source of nutrients for many lakes in Alaska (Donaldson 1967; Kline et al. 1994).

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Coho salmon (*Oncorhynchus kisutch*) spawn and rear in coastal watersheds in North America from central California to northwestern Alaska, often in small streams. Coho salmon, which usually spawn at lower densities than pink and sockeye salmon, are not known as an important vector of nutrients or organic matter from marine to freshwater ecosystems. But because low levels of primary and secondary productivity are characteristic of many headwater streams in the Pacific Northwest (Gregory et al. 1987), even modest inputs of certain nutrients or organic matter of high nutritional value may be of consequence in maintaining trophic productivity.

Previous studies on the impacts of spawning salmon on freshwater ecosystems have suggested that primary production is an important means of uptake of nutrients released by decomposing fish (Richey et al. 1975; Rand et al. 1992; Kline et al. 1994). The small, headwater streams preferred by coho salmon for spawning and rearing typically exhibit low levels of primary production during late autumn and winter because of a combination of heavy shading, low water temperatures, and frequent high flows (Bilby and Bisson 1992). The poor conditions for algal growth suggest that material released by decomposing coho salmon may be incorporated through pathways other than autotrophic uptake.

Stable isotope tracers, such as ^{13}C and ^{15}N, have been used to unravel trophic associations and nutrient dynamics in a variety of terrestrial and aquatic ecosystems (Peterson and Fry 1987). N and C in spawning salmon contain higher proportions of the heavier isotopic form of both elements than N and C imported to streams from other sources (Kline et al. 1990, 1994). Therefore, stable isotope analysis provides a means of tracing the N and C from spawning salmon through the trophic system of the streams they utilize.

We used this technique to investigate the role coho salmon play in the nutrient and trophic dynamics of two small streams in western Washington. Our objectives were to determine the extent to which N or C transported to streams by salmon was incorporated into the tissues of aquatic organisms and riparian plants. We also monitored growth rates of juvenile salmonid fishes to determine if the seasonal availability of the carcasses, eggs, and fry from the spawning fish influenced these parameters. The relative importance of autotrophic, heterotrophic, and chemical uptake of dissolved matter released by the carcasses during decomposition was evaluated by measuring the rate of removal from solution of dissolved organic N derived from carcass tissue by streambed gravel under light and dark conditions and with sterilized gravel. We also monitored dissolved N and P levels in stream water to examine the effect of spawning fish on the concentrations of these elements.

Study sites

We compared N and C isotope ratios among aquatic organisms collected from four tributaries of the Snoqualmie River, located 35 km east of Seattle, Washington, U.S.A. Two of these streams support populations of coho salmon (Grizzly Creek and East Fork Griffin Creek) while access to the other two (an unnamed tributary of Griffin Creek labeled stream 0372 in the Washington stream catalog (Williams et al. 1975) and Ten Creek) is blocked by impassable waterfalls. Thus, stable isotope ratios in samples from the two streams without salmon were produced in the absence of salmon-derived N and C whereas isotope ratios in samples from the streams with salmon represented a mixture of marine and nonmarine N and C sources.

Grizzly Creek and stream 0372 are small (channel width 4–6 m), heavily shaded, second-order streams. The sites exhibit a pool-riffle morphology with a gradient averaging 2%. Overstory vegetation adjacent to the channels is dominated by western hemlock (*Tsuga heterophylla*) and western redcedar (*Thuja plicata*) 80–100 years old, with an understory of salmonberry (*Rubus spectabilis*) and devil's club (*Oplopanax horridum*). Red alder (*Alnus rubra*), a nitrogen-fixing species, was not found along the study reaches of these streams and was a very minor component of the riparian vegetation upstream, on the basis of examination of aerial photographs of the watersheds. Ten Creek and East Fork Creek are third-order streams, slightly larger than the other two but similar in other aspects of channel form. These sites were bordered by a 20 m wide strip of mixed conifer and hardwood trees, which buffered the streams from a clear-cut timber harvest upslope. Red alder was found along these two streams. Annual precipitation in this area averages 150 cm, mostly falling as rain from November through March.

The sites supporting anadromous fishes had more diverse fish communities than the nonanadromous systems. Species captured in East Fork and Grizzly creeks included coho salmon, cutthroat trout (*Oncorhynchus clarki*), steelhead (*Oncorhynchus mykiss*), torrent sculpin (*Cottus rhotheus*), and Pacific lamprey (*Entosphenus tridentatus*). Only cutthroat trout and shorthead sculpin (*Cottus confusus*) were found at the sites inaccessible to anadromous species. Macroinvertebrate communities were similar at all sites, dominated by stoneflies and mayflies.

Estimated abundances of adult coho salmon spawning in East Fork Griffin Creek and Grizzly Creek during the years when samples were collected were 721 fish/km on East Fork Creek during 1990–1991 and 240 and 475 fish/km during 1991–1992 and 1992–1993, respectively, on Grizzly Creek. These estimates are based on fish counts by the Washington Department of Fisheries and the area under the curve method of English et al. (1992), assuming the fish had a 14-day lifespan after entering the study sites.

Methods

Stable isotope ratios are expressed as $\delta^{15}N$ and $\delta^{13}C$ values (Peterson and Fry 1987). These values represent the level of enrichment or depletion of the heavier isotope of N or C relative to a standard. Values are calculated as follows:

$$\delta^{15}N \text{ or } \delta^{13}C = ((R_{sample} - R_{standard})/R_{standard}) \times 1000$$

where R is the ratio of heavy isotope to light isotope. Standards are air for N and Peedee Belemnite for C. Higher values of ^{15}N or ^{13}C indicate higher proportions of the heavier isotope in the sample.

Our initial samples were collected from East Fork Griffin Creek and Ten Creek during February and April 1991. We focused our efforts on Grizzly Creek and stream 0372 during 1992 and 1993 because these sites were better matched in characteristics of the channel and riparian area, had no recent timber harvest in their watersheds, and are in adjacent watersheds with similar drainage areas above the study sites

Table 1. Organisms collected for isotope analysis during this study and their trophic category.

Trophic category	Sample type or organism
Organic matter	Terrestrial: western hemlock needles, *Tsuga heterophylla;* salmonberry leaves, *Rubus spectabilis*; devil's club leaves, *Oplopanax horridum*
	Aquatic: epilithic organic matter
Grazing invertebrates	Ephemeroptera: *Cinygmula* sp., *Epeorus* sp.
Shredding invertebrates	Plecoptera: *Pteronarcella* sp., *Nemoura* sp.
	Diptera: *Tipula* sp.
Collecting-gathering invertebrates	Ephemeroptera: *Paraleptophlebia* sp., *Ephemerella* sp.
Predatory invertebrates	Plecoptera: *Calineuria* sp.
	Odonata: *Octogomphus* sp.
Predatory fishes	Salmonidae: coho salmon, *Oncorhynchus kisutch*; cutthroat trout, *Oncorhynchus clarki*; steelhead trout, *Onchorhynchus mykiss*
	Cottidae: torrent sculpin, *Cottus rhotheus*
Omnivorous fishes	Petromyzontidae: Pacific lamprey ammocoete, *Entosphenus tridentatus*

(Grizzly Creek, 293 ha; stream 0372, 272 ha). Samples were collected at these sites in January, April, October, and December 1992, and March 1993.

Samples collected consisted of foliage from riparian vegetation, epilithic organic matter (the film encrusting rocks on the streambed), aquatic invertebrates, and fishes (Table 1). Samples of riparian vegetation were taken during April 1992 and March 1993. New foliage was picked with forceps and stored in plastic sample bags. Samples of epilithic organic matter were collected by scrubbing the surfaces of stones taken from the streambed with a wire brush. Material dislodged was rinsed with a small volume of stream water into a stainless steel pan and transferred to a glass jar. The samples were subsequently concentrated by centrifugation and filtering onto a pre-ashed glass-fiber filter with a pore size of 1 µm. Epilithic organic matter samples were collected on each sample date.

Invertebrate samples were obtained by disturbing the streambed upstream from a 200-µm mesh net. Material collected was sorted in an enamel pan in the field to remove large invertebrates. Remaining material was placed in a polyethylene bag and transported to the laboratory where it was examined under a dissecting microscope to remove smaller organisms. Invertebrates were sorted by genus. On each sample date, an attempt was made to capture invertebrates representing four trophic categories: shredders, which ingest large pieces of organic matter, usually of terrestrial origin; grazers, which scrape the organic matter from rock surfaces; collector-gatherers, which filter small particles of organic matter from the water column or gather fine particles of algae and detritus from the streambed; and predators, which prey on other invertebrates (Table 1; Merritt and Cummins 1978). However, seasonal scarcity of some invertebrate taxa prevented us from obtaining all trophic groups on all sample dates. Isotope analysis was performed on composite samples of whole invertebrates for each genus. Results are presented as averages for each of the four trophic categories, as diet is the primary determinant of isotope ratios (Minigawa and Wada 1984).

Fish were captured by electroshocking. Dorsal muscle tissue samples were taken from the fish sacrificed for isotope analysis. Dorsal muscle tissue from fresh coho salmon carcasses was collected during February 1991 at East Fork Creek and January 1992 and December 1993 at Grizzly Creek. Salmon eggs found on the streambed surface at Grizzly Creek during January 1992 also were analyzed.

Samples were rinsed first in a 10% solution of HCl to remove any carbonate, and then in distilled water. The samples were dried at 60°C and stored over silica gel desiccant.

Isotope ratio analyses were conducted by Coastal Sciences Laboratories, Austin, Texas. Samples were ground to a powder and combusted to generate CO_2 and N_2 gas. Isotope ratios in the evolved gases were measured with a mass spectrometer (Fison Instruments, Danvers, Mass.). Precision of the analysis (±1 SD), based on replicate samples, was 0.27 ± 0.16 for $\delta^{15}N$ ($N = 36$) and 0.15 ± 0.10 for $\delta^{13}C$ ($N = 13$).

The average size and growth rate of juvenile coho salmon were monitored in Grizzly Creek during 1992–1993 and 1993–1994. The same variables were monitored for age-0 cutthroat trout in stream 0372 during 1992–1993. Fish were collected by electrofishing the study reaches of the two streams during June, October, December, and March. The standard length and weight of each fish captured were measured.

In 1992 and 1993, a fresh coho salmon carcass was collected from East Fork Griffin Creek. These two carcasses were frozen and used to evaluate the uptake by streambed gravel of dissolved organic matter released by decomposing fish. Eight experiments were conducted examining changes in the dissolved Kjeldahl-N and NO_2/NO_3-N (hereafter referred to as NO_3-N) concentration over time in a series of 2-L flasks. These flasks contained combinations of filtered stream water (glass fiber filter; pore size 1 µm), streambed gravel, and salmon muscle tissue extract. The gravel and stream water were collected on the morning that each experiment was conducted. Gravel was sieved in the field into two size-classes, 8–16 and 2–8 mm. A similar gravel surface area in each flask containing gravel was maintained by adding 300 mL of coarse gravel (8–16 mm) and 200 mL of fine gravel (2–8 mm). Sieving may have reduced the amount of epilithic organic matter on the collected gravel. However, it was necessary to minimize variability in gravel surface area among flasks. Each flask contained 500 mL of liquid (combination of stream water and carcass extract).

The carcass extract was prepared by soaking a 25-g chunk of muscle from the middorsal area of the fish in 2 L of filtered stream water for 30 min. The resultant solution was poured through a 200-µm mesh filter to remove larger particulate material and 100-mL aliquots were added to 400 mL of filtered stream water in those flasks that contained extract.

Three combinations were evaluated in each experiment; seven flasks contained filtered stream water and gravel, seven contained filtered stream water and carcass extract, and seven contained stream water, gravel, and extract. Flasks were held in a constant temperature chamber at 6°C, a water temperature

Table 2. $\delta^{15}N$ and $\delta^{13}C$ values for members of different trophic categories in streams with and without salmon.

	Streams with salmon				Streams without salmon			
	Grizzly Creek		East Fork Creek		Stream 0372		Ten Creek	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Coho eggs	16.4±0.1 (2)	−19.6±0.2 (2)						
Coho carcass muscle	14.2±0.2 (2)	−17.9±0.3 (3)	13.0±0.1 (2)	−21.0±0.1 (2)				
Terrestrial vegetation	0.7±1.4 (7)	−31.1±1.5 (5)*			−2.2±2.0 (8)	−30.5±1.3 (5)*		
Epilithic organic matter	7.1±1.6 (5)	−28.2±2.9 (7)			5.2±0.3 (6)	−31.6±1.1 (6)		
Grazers	8.0±1.4 (9)	−27.3±2.0 (10)	8.4±0.1 (2)	−30.1 (1)	5.0±0.3 (6)	−31.5±1.5 (5)	4.2±0.2 (2)	−32.5 (1)
Shredders	4.3±1.9 (9)	−27.1±1.3 (9)*			0.3±0.1 (2)	−27.9±0.6 (3)*		
Collector-gatherers	6.4±0.6 (4)	−26.7±1.5 (6)			4.5±0.7 (3)	−30.7±1.4 (4)		
Invertebrate predators	7.9±1.0 (7)	−26.4±0.8 (6)	9.2±0.5 (2)	−26.8±0.8 (2)	6.4±1.3 (7)	−30.3±1.3 (8)	6.6±0.4 (2)	−30.3±0.6 (2)
Age-0 cutthroat trout	10.0±1.6 (4)	−25.6±1.8 (5)			7.7±0.5 (8)	−28.5±1.5 (6)		
Age-1 and -2 cutthroat trout	10.8±2.2 (6)	−24.0±1.7 (5)			7.6±1.0 (11)	−26.6±1.3 (6)		
Age-1 cutthroat trout			13.6±0.1 (2)	−22.1 (1)			8.1±0.3 (3)	−27.5±1.4 (3)
Age-0 coho salmon	11.5±1.6 (11)	−23.6±1.3 (8)	11.7±0.4 (2)	−25.7±0.3 (2)				
Lamprey ammocoetes	6.4±1.4 (4)	−27.8±1.2 (4)						
Torrent sculpin	10.4±0.2 (3)	−25.6±1.2 (3)						
Age-1 steelhead			11.9±1.4 (3)	−20.6±0.1 (2)				

Note: Values are given as means ± 1 standard deviation with sample size in parentheses.
*Nonsignificant difference between streams with and streams without salmon (t test; $P \leq 0.05$).

commonly occurring when salmon carcasses are present in the study streams. Air pumped through a small pipette was used to maintain circulation and oxygen in each flask. One flask of each combination was removed for analysis at 0, 0.5, 1, 2, 3, 4, and 24 h after initiation of the experiment. Water from the flask was poured into a 500-mL sample bottle containing 1 mL of H_2SO_4 as a preservative and refrigerated until analyzed. Three experiments were conducted with a bank of fluorescent lights illuminating the chamber, three with the lights off and each flask wrapped in black plastic, and two trials in the dark with gravel that had been autoclaved for 30 min.

Samples were filtered and analyzed for dissolved Kjeldahl-N, NH_4-N, and NO_3-N using standard methods (U.S. Environmental Protection Agency 1983).

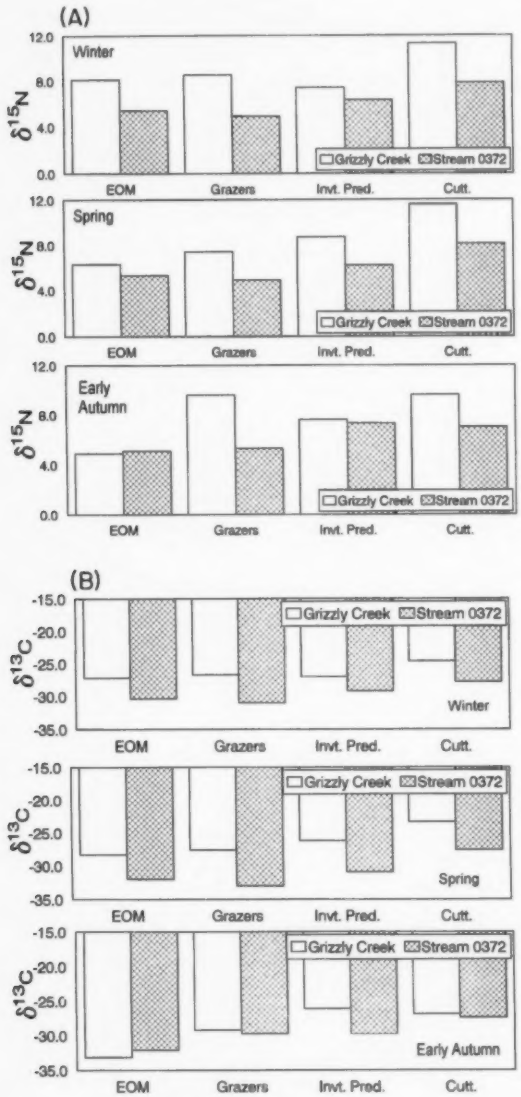
Stream water samples were taken monthly from March 1992 through September 1992 and every 2 weeks from October 1992 through April 1993 from Grizzly Creek and stream 0372. Samples were analyzed for NH_4^+-N, NO_3-N, Kjeldahl-N, and total soluble P using standard methods (U.S. Environmental Protection Agency 1983).

Results

Salmon eggs and muscle from carcasses exhibited the highest $\delta^{15}N$ and $\delta^{13}C$ values of any of the samples collected (Table 2). Eggs were more enriched with the heavier isotope of N and less enriched with the heavier isotope of C than carcass muscle. Coho salmon eggs from Grizzly Creek were enriched with the heavier isotopes of N and C 15.7‰ and 11.5‰, respectively, relative to the foliage from riparian plants along this stream, an important source of terrestrial C and N input to these systems (Bilby and Bisson 1992). Carcass muscle tissue was enriched 13.5‰ for N and 13.2‰ for C relative to terrestrial vegetation. Our ability to distinguish N and C contributed by spawning salmon from that contributed from terrestrial sources was greatly enhanced by this high degree of distinction in isotope ratios.

The differences between the anadromous and nonanadromous sites in $\delta^{15}N$ and $\delta^{13}C$ values averaged for all sample dates (Table 2) were evident even after incorporating variability owing to seasonal changes in isotope ratios at the

Fig. 1. Difference in $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values between Grizzly Creek and stream 0372 for four trophic levels (epilithic organic matter (EOM), grazers, invertebrate predators, and cutthroat trout) during the winter (December and January), spring (March and April), and early autumn, prior to the arrival of spawning salmon (October).



anadromous sites (see below). All $\delta^{15}N$ values for sampl collected from Grizzly Creek were significantly greater than values for the corresponding samples from stream 0372 (t test, $P \leq 0.05$). The same is true of the $\delta^{13}C$ values with the exception of the terrestrial vegetation and shredders. The primary source of C for terrestrial plants is atmospheric CO_2 and shredders ingest terrestrially derived organic matter. It is unlikely that the isotope ratio of atmospheric CO_2 would be influenced by spawning salmon. In all cases where sample size was sufficient to enable a statistical comparison between East Fork and Ten creeks, the values for both $\delta^{15}N$ and $\delta^{13}C$ were significantly higher in the system supporting salmon. Neither shredders nor foliage from terrestrial vegetation were collected at these sites.

Although coho salmon, steelhead, and possibly some of the cutthroat trout we sampled were the offspring of marine adults, the elevated $\delta^{15}N$ and $\delta^{13}C$ levels of these fish were a consequence of dietary intake rather than a chemical legacy from their parents. Recently emerged coho salmon fry collected in March 1993 exhibited a $\delta^{15}N$ value of 14.3 and a $\delta^{13}C$ value of –21.3, reflecting their marine origins. However, the weight of the juvenile fish at emergence from the gravel represents less than 5% of their weight during autumn, winter, and spring, when our samples were collected. The isotope values for newly emerged fry are not included in the average values for juvenile coho salmon presented in Table 2.

For most sample categories, minimum differences between the anadromous and the nonanadromous sites in $\delta^{15}N$ and $\delta^{13}C$ occurred during early autumn, immediately prior to the arrival of spawning coho salmon (Fig. 1). Differences in isotope ratios between the two sites increased during the winter, while carcasses were present in the stream, and remained at high levels through early spring, shortly after the carcasses were fully decomposed and while salmon fry were emerging from the streambed gravel. This pattern was observed for epilithic organic matter, invertebrate predators, and cutthroat trout for N and epilithic organic matter, grazers, and cutthroat trout for C. Differences between the sites in $\delta^{15}N$ values for grazers and in $\delta^{13}C$ values for invertebrate predators did not increase following appearance of the spawning salmon. However, the isotope ratios for these sample categories were higher in the anadromous system at all times.

The weight and growth rate of juvenile coho salmon in Grizzly Creek increased following appearance of spawning salmon in the stream during both 1992–1993 and 1993–1994 (Fig. 2). The mean weight of juvenile coho increased 1.0 and 1.5 g during the summers of 1992 and 1993, respectively. The weight gains during the summer of 1993 were statistically significant (t test; $P \leq 0.05$) but not those observed during the summer of 1992. The fish more than doubled in weight following the arrival of the adult salmon in the system, gaining 3.6 g during the winter of 1992–1993 and 3.2 g during 1993–1994. Changes in weight during both years were significant (t test; $P \leq 0.05$). Rate of growth increased from about 7 mg/fish each day during the summer to 16 mg/fish each day after arrival of the adult salmon in 1992–1993. The following summer growth rates were slightly higher, about 10 mg/fish each day increasing to 13 mg/fish each day in the early winter and to over 20 mg/fish each day in the late winter. Age-0 cutthroat trout in stream 0372 during 1992–1993 displayed a 1.5-g increase in weight over the summer and an additional 1.5 g during the winter. Size changes during both seasons were significant (t test; $P \leq 0.05$). Growth rates of the cutthroat during the winter months were similar to or less than the rates seen over the summer. Insufficient numbers of cutthroat trout were captured in Grizzly Creek to examine their growth response to the availability of salmon carcasses.

Streambed gravel rapidly removed Kjeldahl-N from solution in our uptake experiments (Fig. 3). Background stream

Fig. 2. Seasonal changes in weight of age-0 coho salmon from Grizzly Creek for 1992–1993 and 1993–1994 and age-0 cutthroat trout from stream 0372 for 1992–1993, and the rate of daily biomass accumulation necessary to achieve these weight gains. June–October includes through October 15; October–December includes through December 15. Spawning salmon and carcasses are present in the stream from late October through late February. Weights are shown + one standard deviation.

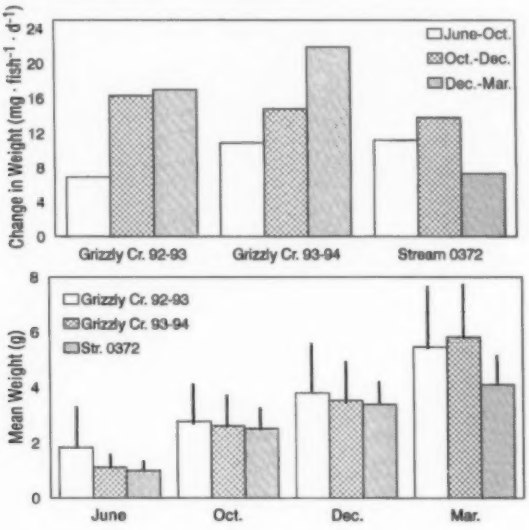
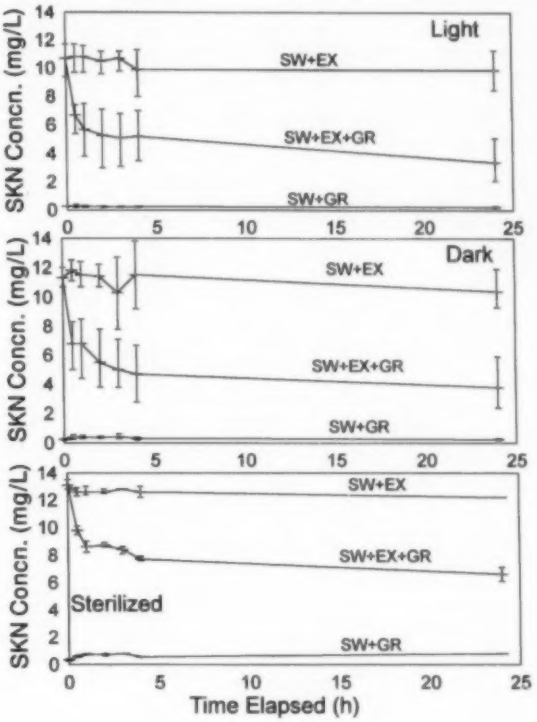


Fig. 3. Change in soluble Kjeldahl-N (SKN) concentration over time in flasks containing stream water and streambed gravel (SW + GR), stream water and carcass extract (SW + EX), and stream water, extract, and gravel (SW + EX + GR). Each point displays the mean and range of concentration values obtained. Three experiments each were conducted under light and dark conditions, and two with sterilized gravel.



water concentrations of Kjeldahl-N were less than 1 mg/L in all experiments. Addition of the carcass extract elevated Kjeldahl-N concentrations in our light and dark experiments to approximately 11 mg/L. In flasks containing extract, gravel, and stream water, under both light and dark conditions, removal of Kjeldahl-N from solution proceeded rapidly for the first several hours, with concentrations decreasing on average 5.4 and 6.6 mg/L after 4 h in the light and dark experiments, respectively. Concentrations decreased an additional 1.8 mg/L in the light treatment and 0.8 mg/L in the dark treatment by 24 h. There was no significant difference in Kjeldahl-N concentration between the light and dark experiments (ANOVA: $P \leq 0.05$). There was no change in Kjeldahl-N concentration over time in either the flasks with gravel and stream water or those with extract and stream water.

Kjeldahl-N uptake experiments conducted with sterilized gravel exhibited a similar pattern of rapid uptake initially followed by slower uptake after several hours (Fig. 3). The flasks with the extract exhibited slightly higher starting concentrations of Kjeldahl-N than those for the light and dark experiments. However, the total change in concentration was only slightly less than that observed for untreated gravel, with average decreases of 5.3 mg/L after 4 h and 6.5 mg/L after 24 h.

Addition of the extract had no effect on the initial concentration of NO_3-N (Fig. 4). NO_3-N levels increased slowly during incubation in the flasks containing stream water, extract, and gravel in both the light and dark experiments, increasing 0.5 to 0.6 mg/L over 24 h. Differences between the light and dark treatments were not significant (ANOVA: $P \leq 0.05$). No increase in NO_3-N concentration was observed in the flasks containing stream water and extract and a slight increase (0.1–0.2 mg/L) was seen in the flasks containing gravel and stream water. None of the experiments conducted with sterilized gravel exhibited an increase in NO_3-N concentration, reflecting the elimination of nitrifying bacteria.

The stream water concentration of NO_3-N in Grizzly Creek increased in autumn (Fig. 5). Concentrations remained elevated through spring. Stream 0372 did not exhibit an increase in NO_3-N concentration during the autumn and winter. Kjeldahl-N concentrations in the two streams were similar and displayed no obvious seasonal trend (Fig. 5). Higher concentrations of NH_4^+-N and total soluble P in both streams occurred during winter. However, there were no differences in either seasonal pattern or concentration between the two streams. NH_4^+-N concentrations ranged from below detection levels (0.01 mg/L) to 0.07 mg/L in Grizzly Creek and from below detection levels to 0.05 mg/L in stream 0372. Total soluble P concentrations ranged from 0.01 to 0.09 mg/L in Grizzly Creek and from 0.01 to 0.08 mg/L in stream 0372.

Table 3. Values for $\delta^{15}N$ and $\delta^{13}C$ assuming a diet based only on organic matter from spawning coho salmon.

Sample category	$\delta^{15}N$	$\delta^{13}C$
Terrestrial vegetation	14.1	
EOM	14.1	−18.1
Primary consumers	17.1	−17.1
Secondary consumers	20.1	−16.1

Note: Values for terrestrial vegetation and epilithic organic matter (EOM) were assumed to be equal to the values exhibited by the salmon tissues sampled from Grizzly Creek. Fractionation rates used for primary and secondary consumers were 3.0‰ for N and 1.0‰ for C.

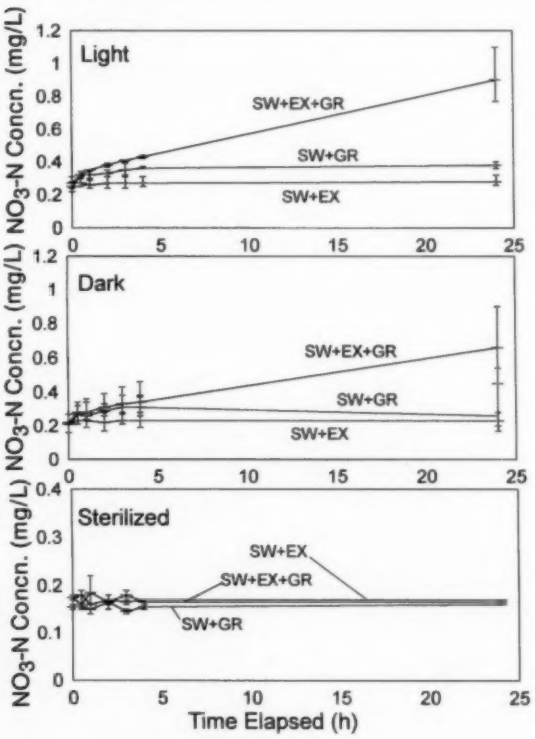
Discussion

We estimated the proportions of N and C contributed by spawning salmon to the biota at our study sites by determining the amount of heavier isotope needed to elevate $\delta^{15}N$ and $\delta^{13}C$ values in the streams without salmon to levels found in the corresponding sample category in the streams supporting salmon (Kline et al. 1990). We have assumed that the sources of N and C and the isotope ratios of these sources are similar for the streams we examined, except for the spawning salmon. Given the proximity and similarity in physical characteristics and vegetation among these streams, we feel this assumption is warranted.

Determination of the contribution of N and C from spawning salmon requires estimation of isotope ratios for each trophic level if only salmon-derived N and C were available (Table 3; Kline et al. 1990). Stable isotope ratios for N and C change as a result of trophic exchanges, a process called fractionation (DeNiro and Epstein 1978; Minigawa and Wada 1984; Peterson and Fry 1987). We assumed that no fractionation occurred in the uptake of N and C by epilithic organic matter. Thus, the $\delta^{15}N$ and $\delta^{13}C$ values for epilithic organic matter were assumed to equal the average for salmon eggs and carcasses (Table 2).

Some isotopic fractionation during uptake of N and C by epilithic organic matter might be expected. Isotopic fractionation is usually associated with C uptake by autotrophs (Peterson and Fry 1987). However, epilithic organic matter is a mix of algae, fungi, and bacteria in a polysaccharide matrix (Lock et al. 1984) and typically exhibits $\delta^{13}C$ values much higher than those for pure algae from the same stream (Rosenfeld and Roff 1992). In N-limited systems bacterial or autotrophic uptake of N usually occurs without isotopic fractionation (Peterson and Fry 1987). However, Grizzly Creek exhibits dissolved N concentrations over 2.0 mg/L at times during the winter, suggesting that the system may not be N limited at this time of year (Fig. 5). Our uptake experiments indicate that the primary pathway of salmon-derived N and C incorporation into epilithic organic matter was by chemical sorption rather than autotrophic uptake. Therefore, fractionation rates would be less than would occur if autotrophic uptake were the predominant mode of incorporation, but some fractionation of both N and C may occur. However, isotopic fractionation of C during uptake by autotrophs or isotopic fractionation of N in systems where this element is abundant favors the lighter isotope

Fig. 4. Change in NO_3-N concentration over time in flasks containing stream water and streambed gravel (SW + GR), stream water and carcass extract (SW + EX), and stream water, extract, and gravel (SW + EX + GR). Each point displays the mean and range of concentration values obtained. Three experiments each were conducted under light and dark conditions and two with sterilized gravel. Note that the *y* axis of the plot for sterilized gravel has a different scale than the plots for light and dark conditions.

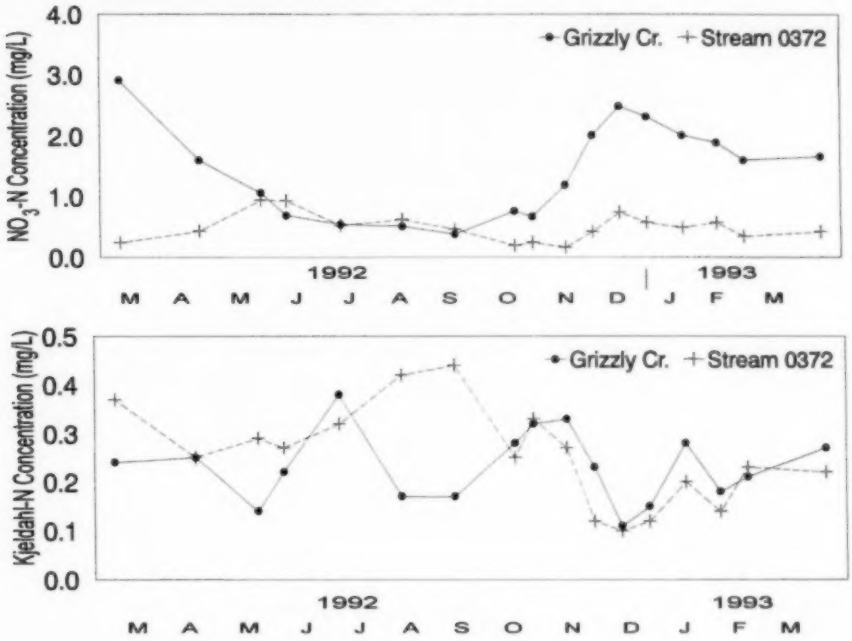


(Peterson and Fry 1987). Any fractionation occurring during incorporation of marine N and C by epilithic organic matter would make our estimates of the proportion of N and C contributed by spawning salmon (Table 4) low.

Fractionation rates of 3.0‰ were used for N for trophic exchanges involving primary and secondary consumers (Minigawa and Wada 1984; Kline et al. 1990) and 1.0‰ for C (DeNiro and Epstein 1978). In generating the isotope ratio values in Table 3 we assumed that fish fed only on invertebrates, ignoring the fact that salmon eggs, carcass tissue, and fry are ingested when they are available. Direct consumption of these materials would lower the values for $\delta^{15}N$ and $\delta^{13}C$ for fish and cause our estimates of the proportion of salmon-derived N and C for fish (Table 4) to be low. Thus, the accuracy of the assumptions applied in estimating contributions of N and C from spawning salmon is unlikely to alter our conclusion that spawning coho salmon made a substantial contribution of N and C to the biota of our study sites.

Contribution of N by spawning salmon to the biota of Grizzly Creek, averaged for all sample dates, ranged from a

Fig. 5. NO_3-N and dissolved Kjeldahl-N in stream water in Grizzly Creek and stream 0372 for the period from March 1992 through March 1993.



low of 10.9% for invertebrate predators to a high of 30.6% for juvenile coho salmon (Table 4). The foliage of riparian plants along Grizzly Creek contained 17.5% salmon-derived N. Except for riparian foliage and shredders, salmon-derived C estimates were very similar to those for N, ranging from a low of 25.2% for epilithic organic matter to a high of 39.5% for juvenile coho. Proportions of salmon-derived N for samples from East Fork Creek tended to be higher than those obtained for Grizzly Creek whereas the C values were more variable.

The highest proportions of salmon-derived N in the biota of Grizzly Creek for any sample date ranged from a low of 18.0% for invertebrate predators to 44.8% for older cutthroat trout; corresponding values for C ranged from 26.6% for epilithic organic matter to 44.0% for coho salmon (Table 4). All peak proportions of marine N and C in the biota were seen during the winter and spring (Fig. 2), during and shortly after spawning by coho salmon.

It generally has been assumed that nutrients from spawning salmon are introduced into the trophic systems of streams through uptake by primary producers after mineralization to inorganic forms (Richey et al. 1975; Kline et al. 1990; Rand et al. 1992). However, uptake of salmon-derived N and C through autotrophic production would be limited in our study streams by low light levels, short day length, cold temperatures, and frequent scouring flows at the time when carcasses are present. Winter gross primary production in a stream approximately 50 km south of the study sites was 15% of the rate measured during summer (Bilby and Bisson 1992).

Our results suggest that pathways other than autotrophic uptake are primarily responsible for incorporation of salmon-derived organic matter into the trophic systems of the streams we examined. All aquatic organisms in the salmon-bearing streams we sampled, except shredders, exhibited significant enrichment with both ^{15}N and ^{13}C, suggesting that incorporation of marine-derived nutrients into the stream biota occurs while the N and C are in an organic form, prior to mineralization.

One method of incorporation was consumption of carcasses, eggs, and emerging fry by fish, and possibly by some of the invertebrates. However, the presence of large amounts of salmon-derived N and C in epilithic organic matter and invertebrates that were unlikely to have fed directly on carcasses, eggs, or fry indicated that other processes of incorporation also were operating. Our laboratory experiments indicated that dissolved Kjeldahl-N derived from carcasses was rapidly removed from solution by streambed gravel. No uptake was observed in the absence of gravel. Uptake rates under both light and dark conditions also were similar, indicating that autotrophic processes were of minimal importance in the incorporation of marine organic matter. Our experiments suggest that uptake is largely an abiotic process, as the rate of removal of dissolved Kjeldahl-N from solution was only slightly higher for sterilized than unsterilized gravel. Chemical uptake of dissolved organic matter by streambed substrate is well documented (Lock and Hynes 1976; Dahm 1981; Fiebig and Marxsen 1992).

Care must be taken in extrapolating the results of the flask experiments to the field. Gravels used in these experiments were disturbed by sieving, transport to the laboratory, and autoclaving. The characteristics and rates of uptake may have been altered as a result of this disturbance. In addition, the short incubation times do not indicate the ultimate fate of the

Table 4. Proportion of salmon-derived N and C in the biota of the anadromous streams.

	Grizzly Creek					
	Average values		Peak values		East Fork Creek	
	% marine N	% marine C	% marine N	% marine C	% marine N	% marine C
Riparian foliage	17.5	0	—*	—*		
Epilithic organic matter	20.7	25.2	30.4	26.6		
Grazers	24.8	29.2	36.4	33.9	32.6	15.6
Shredders	23.8	0	—*	—*		
Collector-gatherers	14.43	29.4	20.8	33.1		
Invertebrate predators	10.9	27.5	18.0	31.8	19.2	24.6
Age-0 cutthroat trout	18.5	23.4	38.2	36.8		
Age-1 and -2 cutthroat trout	25.6	24.8	44.8	36.2		
Cutthroat Trout					45.8	47.4
Age-0 coho salmon	30.6	39.5			30.0	15.7
Age-1 steelhead					31.7	60.5

Note: Average values are calculated from all samples collected on all dates. Values for coho salmon and steelhead assume they would exhibit the same $\delta^{15}N$ or $\delta^{13}C$ values as cutthroat trout in streams without salmon if no N or C from spawning salmon were available. The peak values represent the maximum difference in $\delta^{15}N$ or $\delta^{13}C$ seasonally (autumn, winter, spring) between the anadromous and nonanadromous streams. Sampling frequency at Ten Creek and East Fork Creek was insufficient to enable determination of seasonal peaks in proportion of salmon-derived N and C.

*Sample types for which a sample from one of the seasonal categories was missing.

material adsorbed onto the gravel surfaces. However, these experiments do demonstrate that streambed gravel can rapidly remove dissolved compounds released by salmon carcasses from solution and that abiotic uptake plays a key role in this process.

The fact that incorporation of salmon-derived N and C into the trophic system of streams occurs primarily either through direct consumption by secondary consumers or by sorption onto the streambed indicates that spawning salmon may be important in maintaining production regardless of the relative availability of nutrients from other sources. Because uptake by primary producers is not necessary for the introduction of spawner-derived organic matter and nutrients into the trophic system, the relative availability of nutrients controlling plant growth should not greatly influence the rate of incorporation of N and C from spawning salmon. Rather, the rate of incorporation of organic matter provided by the salmon into the system would be related to its nutritional quality relative to other sources of organic matter available in the stream.

The effect of spawning salmon on the stream water chemistry of Grizzly Creek was not conclusive. We observed increases in NO_3-N during the time of spawning, which is consistent with our observation that NO_3-N concentration increased in the flasks with gravel, stream water, and extract after several hours of incubation. However, previous studies also have reported increases in Kjeldahl-N and NH_4-N associated with spawning salmon (Brickell and Goering 1970). We saw no seasonal changes in Grizzly Creek nor was there any difference in concentration between Grizzly Creek and stream 0372 during spawning for Kjeldahl-N, NH_4-N, or total soluble P. The lack of change in concentration suggests that dissolved materials released by the decomposing fish were rapidly removed from the water. We cannot determine the extent to which the decomposing carcasses contributed to the increase in NO_3-N concentration in Grizzly Creek.

The high proportions of marine-derived N and C in the stream biota and the increase in the growth rate of juvenile fish corresponding to the appearance of spawning salmon indicate that spawning coho salmon are an ecologically significant source of organic matter and nutrients for the stream systems we examined. In contrast, winter growth rates of age-0 cutthroat trout in stream 0372 were either similar to or lower than at other times of the year. Increased growth rate may have consequences later in life. Larger size has been shown to substantially increase the over-winter survival rate of juvenile coho salmon (Hartman and Scrivner 1990) and larger smolt size has been related to increased marine survival in both coho salmon (Bilton et al. 1982) and steelhead (Ward and Slaney 1988). Reductions in winter and marine survival will reduce the number of returning adult salmon and further suppress the productivity of the freshwater system, thus creating a feedback process that progressively depresses the capacity of the freshwater system to support fish.

Populations of wild coho salmon have declined precipitously over the last several decades in Washington, Oregon, and California (Nehlsen et al. 1991; Nickelson et al. 1992; Washington Department of Fisheries 1993). Many factors have contributed to the decline, including overfishing, alteration and loss of freshwater and estuarine habitat, reduced ocean productivity, inappropriate hatchery practices, introduction of exotic species, and development of dams and other water projects (Nehlsen et al. 1991; Pearcy 1992). In addition, a decline in the number of returning salmon, and thus in the nutrients they carry, has consequences for the productivity of stream ecosystems. Large-scale declines of coho populations in many coastal watersheds may represent significant losses of trophic productivity and nutrient capital in headwater streams, and reduced export of nutrients and organic matter from these streams may affect production in aquatic ecosystems downstream from areas in which spawning occurs (Vannote et al. 1980). The influence of spawning salmon on the trophic productivity of small headwater streams may be especially

important because heavy shading limits autotrophic production throughout the year (Gregory 1980). Decreased productivity may be self-perpetuating if the capacity of a watershed to produce future generations of coho salmon is progressively diminished because there are fewer and fewer returning adults.

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Paleolimnological reconstructions of long-term changes in fisheries status in Sudbury area lakes

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Abstract: Long-term fisheries data are often lacking for small lakes. This is certainly true in Ontario's Sudbury region, where many lakes currently lack fish, presumably because of impacts from local mining and smelting operations. If rehabilitation of such lakes is being considered, it is prudent to have knowledge of their past fisheries status. We assessed the fisheries history of some lakes in the Sudbury region using the sedimentary remains of *Chaoborus* larvae. Among six recently fishless lakes, Bat Lake is a bog that apparently has never supported fish populations, and data were inconclusive for Ruth-Roy Lake. In Swan, Hannah, Middle, and Clearwater lakes, fish apparently disappeared during either the 1940s or 1950s when pH levels were declining and metal concentrations were increasing. Hannah and Middle lakes were limed in the mid-1970s, and pH levels have remained circumneutral. Fish have since recolonized both lakes. Crowley Lake currently supports fish; it apparently always supported fish, and no conclusion could be made as to whether its yellow perch (*Perca flavescens*) were introduced. Where total fish losses were indicated, the disappearances coincided with pH values ≤ 5.0 inferred from diatom and chrysophyte analyses, levels at which fish reproduction is usually unsuccessful or severely depressed.

Résumé : On manque souvent de données à long terme sur les pêches dans les petits lacs. C'est certainement le cas dans la région de Sudbury, en Ontario, où les poissons se font rares dans de nombreux lacs, ce qui est probablement causé par les effets des activités locales d'extraction minière et de fonderie. Si on envisage de remettre ces lacs en état, il est prudent d'avoir des renseignements sur l'histoire de la pêche qui s'y est pratiquée. Nous avons essayé de reconstituer l'histoire de la pêche dans certains lacs de la région de Sudbury à partir des restes de larves de *Chaoborus* présents dans les sédiments. Parmi six lacs vides de poisson ces derniers temps, le lac Bat est une tourbière oligotrophe qui ne semble avoir jamais abrité de populations de poissons, et les données n'étaient pas concluantes en ce qui concerne le lac Ruth-Roy. Dans les lacs Swan, Hannah, Middle et Clearwater, il semble que les poissons aient disparu dans les années 40 ou 50, lorsque le pH a baissé et que les concentrations de métaux ont augmenté. Les lacs Hannah et Middle ont été chaulés au milieu des années 70, et le pH y est demeuré à peu près neutre. Depuis, les poissons ont recolonisé ces deux lacs. À l'heure actuelle, le lac Crowley abrite du poisson; il semble avoir toujours été poissonneux, et on ne peut tirer de conclusion quant à la possibilité que les perchaudes (*Perca flavescens*) qu'il abrite aient été introduites. Aux endroits où les poissons ont complètement disparu, les disparitions ont coïncidé avec des pH ≤ 5,0, d'après l'étude des diatomées et des chrysophytes; à ces niveaux, la reproduction des poissons est généralement impossible ou gravement affaiblie.
[Traduit par la Rédaction]

Introduction

Long-term records of lake fisheries are often lacking for most smaller lakes. For example, it is generally known that many presently acidic lakes are fishless, but relatively little historical data are available to determine when these lakes lost their fish populations, or if they are naturally fishless. Knowledge of past fisheries, however, has important management implications (Uutala et al. 1994). Naturally fishless lakes are not good candidates for stocking programs, and an understanding of the environmental thresholds required to eliminate fish populations can assist in setting critical load guidelines.

In the absence of historical data, indirect proxy methods must be used to infer past changes in fisheries. Paleolimnology, the branch of aquatic science that uses the physical, chemical, and biological records preserved in dated sediment profiles, allows the reconstruction of past environmental conditions (Smol 1992). Algal indicators, such as diatoms (Dixit et al. 1992) and chrysophytes (Smol 1995), have been used extensively to infer past trends in water chemistry, such as lake-water pH. Reconstructing past fishery trends is more problematic, because fish fossils are notoriously rare in lake sediments. Indirect inference, however, can be made using invertebrate indicators (Uutala 1990).

The chitinous mandibles of *Chaoborus* (phantom midge)

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larvae preserved in sediment cores have been used to infer past fisheries trends (Johnson et al. 1990; Uutala 1990; Kingston et al. 1992; Uutala et al. 1994). As reviewed in the above papers, the five main *Chaoborus* species that live in northeastern North America differ in their migratory habits, size, and coloration, characteristics that affect their susceptibility to fish predation (Borkent 1981). *Chaoborus punctipennis*, *Chaoborus albatus*, *Chaoborus flavicans*, and *Chaoborus trivittatus* all exhibit diurnal vertical migrations and can usually co-exist with fish, although the latter taxon is usually dominant in lakes with relatively few species of fish. *Chaoborus americanus* is nonmigratory and easily preyed upon by fish, and so the presence of this taxon's mandibles in sedimentary profiles can be used to indicate the absence of fish (Uutala et al. 1994).

Uutala (1990), Johnson et al. (1990), Kingston et al. (1992), and Uutala et al. (1994) used the above paleolimnological approaches to track past fisheries declines in lakes affected by acidic deposition. Meanwhile, Miskimmin and Schindler (1994) used fossil *Chaoborus* mandibles to trace the effects of toxaphene treatments on past manipulations of fish communities, and Lamontagne and Schindler (1994) used fossil mandibles to determine if fish were present in several Rocky Mountain lakes prior to fish stocking that occurred early in this century.

This paper examines *Chaoborus* mandible distributions in the sediments of a suite of lakes that have previously been studied using a variety of paleolimnological approaches, such as diatom and chrysophyte analyses, in the Sudbury region of Ontario (Dixit et al. 1987, 1989*a*, 1989*b*, 1990, 1992). Sudbury is a major nickel mining and smelting region and remains one of the largest point sources of sulphur pollution in the world. Not surprisingly, a large number of lakes in this region have been affected by acid and metal pollution (Beamish and Harvey 1972; Keller et al. 1986). Many of the lakes are currently fishless, but the history of fish losses, if in fact the lakes ever had fish, is unknown. In this paper we use paleolimnological techniques to show that most of the presently fishless lakes have lost their fisheries concurrently with lake acidification.

Study area

All of the lakes are located on the Canadian Shield in the Sudbury region of Ontario, except for Bat Lake, which is located in Algonquin Park to the southeast of Sudbury. Swan, Hannah, Middle, and Clearwater lakes are all within the city limits of Sudbury and are relatively close to the smelting operations. All four lakes were acidic, had elevated metal concentrations, and sustained no fish populations in the 1970s (Keller et al. 1980; Yan and Miller 1984; Dillon et al. 1986; Poulin et al. 1990). Clearwater (46°22′N, 81°03′W) and Swan (46°22′N, 81°04′W) lakes are still acidic and lack fish, though there are signs that water quality is improving (Yan and Lafrance 1984; Dillon et al. 1986; McIsaac et al. 1986; Poulin et al. 1990). Hannah (46°26′N, 81°02′W) and Middle (46°23′N, 81°06′W) lakes were both treated with neutralizing agents and fertilizers in the mid-1970s (Ontario Ministry of the Environment 1982; Yan and Lafrance 1984; Poulin et al. 1990). The pH levels in Hannah and Middle lakes remained high (about 6.5) following the treatments, but metal concentrations remained at toxic levels. The catchments of Middle and Hannah lakes were limed, fertilized, and seeded as part of the Greening of Sudbury initiative in the early 1980s. These activities, coupled with the decline in emissions from local industries, have resulted in declines in metal levels in Middle and Hannah lakes during the 1980s, and several species of fish have since recolonized both lakes (Poulin et al. 1990).

Crowley Lake (46°23′N, 80°59′W) is also located within the city limits, near its southern edge, but it is not acidic. It is of interest to this study because government scientists suspected that its yellow perch population may have been recently introduced to the lake. Knowledge of whether or not this is so would enable fisheries managers to assess the current productivity and viability of the perch population.

Ruth-Roy Lake (46°06′N, 81°15′W) is farther to the south, in Killarney Provincial Park. It is currently acidic and devoid of fish (Polkinghorne and Gunn 1981). There is paleolimnological evidence that Ruth-Roy is somewhat naturally acidic (Dixit et al. 1992). Bat Lake, located in Algonquin Provincial Park (45°35′N, 78°31′W), is a bog lake with a pH of about 4.6 (Hoeniger 1986).

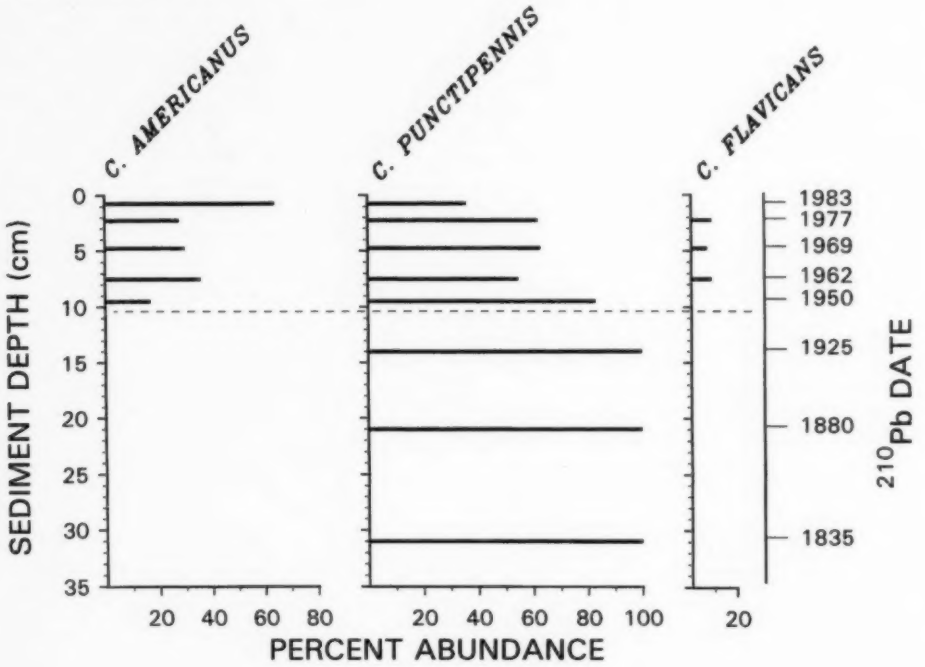
Methods

Core samples were taken from near the deepest point of each lake using a modified Kajak–Brinkhurst type core sampler with a Plexiglas core tube of 6.5 cm inside diameter (Glew 1989). Sectioning of the cores varied, with finer resolution sectioning during the lakes' most recent histories. The top 5 cm of each core were sectioned into either 0.25- or 0.5-cm sections, the next 5 cm into either 0.5- or 1.0-cm sections, and the remainder of the core (below 10 cm) into either 1.0- or 2.0-cm sections. All sectioning was done using the vertical extruder and sectioner designed by Glew (1988).

Generally 3 to 5 mL of wet sediment per sample was prepared for *Chaoborus* analyses, using methods slightly modified from Warwick (1980). Samples were deflocculated in 8–10% (w/v) KOH at room temperature for 2–3 days. The samples were then sieved on 70-μm mesh. The material retained on the sieve (constructed from translucent plastic canisters for 35-mm film) after being washed with distilled water, was dehydrated in a series of ethanol baths (70, 95, and 100%). The retained material was then transferred to glass vials using 100% ethanol for storage. *Chaoborus* mandibles were sorted from the retained material in glass dishes at a magnification of 25–50× under a stereomicroscope. The material was sorted in 100% ethanol and the material was kept just moist in a discrete pile in the center of the dish. Discarded material was pushed to the outer edges of the dish. The much lower surface tension of ethanol (as compared with water) greatly eases the sorting and picking of *Chaoborus* remains. The collected mandibles were mounted in Euparal® on microscope slides and identified following Uutala (1990). *Chaoborus punctipennis* and *C. albatus* are closely related species, and their mandibles cannot be separated (Uutala 1990). *Chaoborus punctipennis* is much more common in the study region than *C. albatus* (Yan et al. 1985) and the mandibles of their type are reported herein as *C. punctipennis*.

^{210}Pb dating of selected cores was done using the methods of Eakins and Morrison (1978), as modified by Cornett et al.

Fig. 1. Chaoborus profile from Swan Lake. Tick marks on the ^{210}Pb date axis indicate the samples that were analyzed. Broken line marks the inferred onset of acidification (Dixit et al. 1989*b*). Numbers of mandibles per sample: 11–32 in the top four samples, 6 in the 1950 sample where *C. americanus* first occurred, and 4–8 in the bottom three levels.



(1984). Profiles of ^{210}Pb activity were determined at the Chalk River Nuclear Laboratory of Atomic Energy Canada Ltd. (Chalk River, Ont.). The age of each section was calculated using the computer program of Binford (1990), which assumes a constant rate of supply of ^{210}Pb to the sediments.

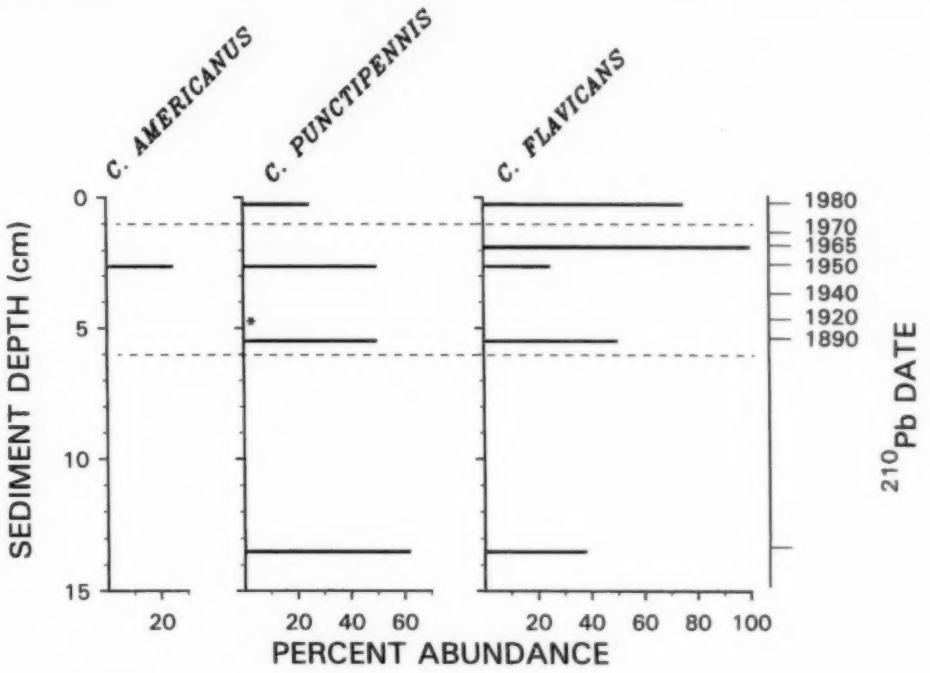
Results and discussion

Chaoborus assemblages at the bottom of cores from both Swan Lake (Fig. 1) and Hannah Lake (Fig. 2) consist of migratory species, primarily *C. punctipennis*, whereas *C. americanus* occurs near the tops of the cores. In the Swan Lake core, the appearance of *C. americanus* in the 9- to 10-cm interval indicates that fish disappeared by the early 1950s (Fig. 1). Swan Lake is currently fishless and paleolimnological data indicate that the lake began acidifying from a background pH between 5.5 and 6.0 by the 1950s, though some recent recovery in pH has been inferred (Dixit et al. 1989*b*). Meanwhile, Keller et al. (1992), using measured limnological data, reported on the recent reacidification of Swan Lake and subsequent pH fluctuations that may be related to climatic factors. Thus, recolonization of the lake by fish likely will not be possible until the pH of Swan Lake recovers completely and stabilizes at its preacidification level.

The appearance of *C. americanus* in the Hannah Lake core indicated that fish disappeared at least by 1950 (Fig. 2) after being apparently present during the 1800s. Despite the low numbers of mandibles in some samples, the apparent loss of all fish populations is clear, but the timing of that is uncertain. The fish losses could have occurred earlier than 1950, perhaps by the early 1900s. Diatom analyses indicated that the lake began acidifying ca. 1900 (Dixit et al. 1987). Assessments of past metal concentrations in the Hannah Lake core sediments indicate that copper and nickel deposition also increased ca. 1900, but that aluminum levels did not increase until the 1940s and 1950s (Dixit et al. 1989*a*), which more closely coincides with the fish loss indicated by *C. americanus*. The inferred preacidification pH of Hannah Lake was around 6.0. The inferred fish loss coincides with a time when the inferred pH dropped below about 5.1, which is when aluminum would be increasingly mobilized from the sediments, and copper and nickel would have been abundant. Hannah Lake was limed in 1975 and yellow perch and minnows have successfully recolonized the lake, with yellow perch being the dominant species by far (Poulin et al. 1990); *C. americanus* was absent from the level of the core deposited after liming and recolonization by fish.

Clearwater Lake has been fishless at least since the 1970s (Polkinghorne and Gunn 1981; Poulin et al. 1990). Diatom and chrysophyte paleolimnological analyses indicated that the lake acidified after ca. 1930–1940 (Dixit et al. 1987). The *Chaoborus* profile (data not shown) indicated that *C. americanus* was present only in the level representing 1965, indicating the lake was fishless by then. Unfortunately, mandibles were extremely rare in the core and were absent from most intervals, and so it remains uncertain how long the lake has

Fig. 2. *Chaoborus* profile from Hannah Lake. Tick marks on the ^{210}Pb axis indicate the samples that were analyzed. The lower broken line marks the inferred onset of acidification, and the upper broken line the inferred pH recovery following liming (Dixit et al. 1987). The number of mandibles per sample was variable and relatively low. No mandibles were recovered from the 1920, 1940, and 1970 samples. A single antennal fragment from the 1920 sample indicated the presence of *C. punctipennis*, indicated by the asterisk. Thirteen mandibles were recovered from the bottom-most sample at 13–15 cm, and 2 to 8 were recovered from the other samples.



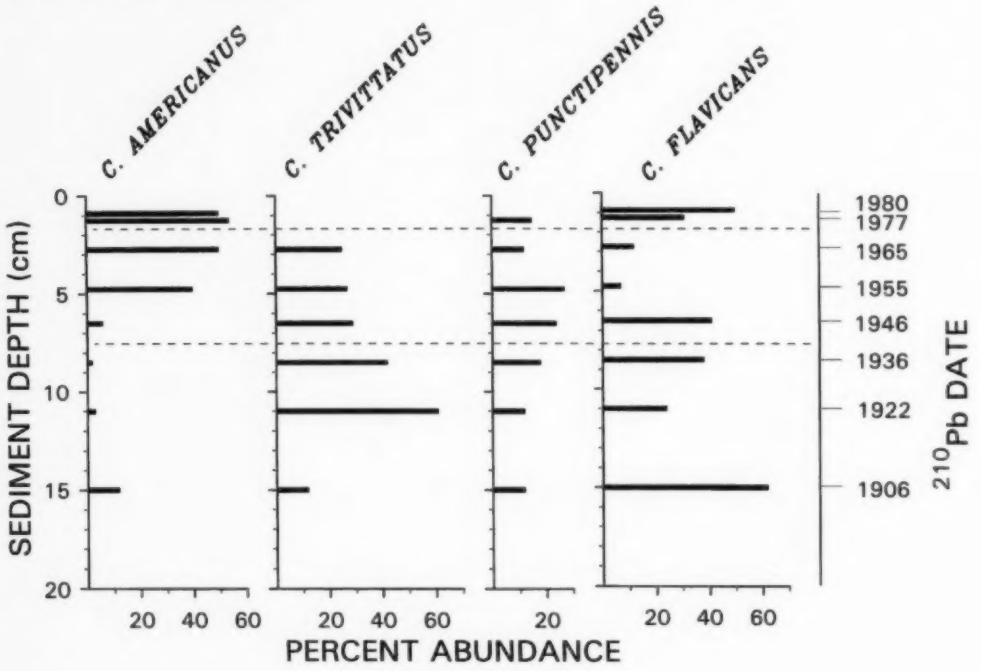
been fishless. This rarity is not surprising, because Yan et al. (1985) reported *Chaoborus* abundance in Clearwater Lake to be quite low.

The Middle Lake diatom and chrysophyte flora has undergone two major changes in its recent history. The first major diatom change occurred between ca. 1935 and the mid-1970s, when taxa characteristic of more acidic waters increased in relative abundance, indicating lake acidification (Dixit et al. 1990). This coincides with the sharp rise in abundance of *C. americanus* in the 1950s (Fig. 3). *Chaoborus americanus*, however, was present in low numbers prior to the 1950s. Uutala et al. (1994) found *C. americanus* at low abundance in a few lakes with only one or two species of fish present at low abundance. This indicates that the preacidification community of planktivorous fishes in Middle Lake was probably simple, similar to those reported by Uutala et al. (1994). The rise in relative abundance of *C. americanus* marks the loss of those fishes from the lake.

The second species shift among the diatoms and chrysophytes occurred soon after Middle Lake was neutralized in 1973 when circumneutral and alkaliphilic taxa became more common (Dixit et al. 1990). The lake was stocked with smallmouth bass, Iowa darters, and sticklebacks in 1976, but apparently none of these fish survived because metal concentrations in the lake water remained high (Yan and Lafrance 1984). *Chaoborus americanus* remained dominant through the 1970s (Fig. 3), occurring in the top two levels that were analyzed representing estimated dates of 1977 and 1980. Younger sediment was not available for analysis. Several species of fishes, predominantly yellow perch, recolonized the lake during the 1980s following a drop in metal levels in the lake. The lower metal concentrations were probably a result of both declining emissions from local smelting industries and watershed liming done in 1983 (Yan and Lafrance 1984; Poulin et al. 1990). The decline in the chrysophyte *Mallomonas pseudocoronata* reported by Dixit et al. (1990) in the 1980s coincides with the decline in metal levels in the lake.

Crowley Lake was included in this study because although the lake presently contains fish, local fisheries managers were uncertain whether the yellow perch in the lake had been introduced recently (J. Gunn, Ontario Ministry of Natural Resources, Sudbury, Ont., personal communication). *Chaoborus americanus* was absent in the sedimentary profile (Fig. 4), so there is no evidence that the lake was devoid of fish. Given the dominance of *C. punctipennis* and *C. flavicans* in all levels of the core, there is no conclusive evidence as to whether or not perch were recently introduced to the lake. The pair of *C. punctipennis* and *C. flavicans* make up the most common *Chaoborus* assemblage in Ontario lakes (Yan et al. 1985). The decline in the relatively rare *C. trivittatus* in the top few

Fig. 3. *Chaoborus* profile from Middle Lake. The lower broken line marks the inferred onset of acidification and the upper broken line the inferred pH recovery following liming (Dixit et al. 1990). Sediment was scarce in the topmost sample and only 2 mandibles were recovered, the three next older samples had 8–17 mandibles, and the bottom three samples had 16–74 mandibles.



centimetres of the core could be related to an introduction of perch sometime during the past 20–40 years, but this cannot be stated with any certainty.

Chaoborus americanus mandibles represented 79–88% of the *Chaoborus* fossils (range of numbers per sample was 14–35) recorded at all levels of the Bat Lake core, indicating that this lake has been fishless for at least the last 200 years. The other mandibles, predominantly *C. trivittatus*, and *C. punctipennis*, occurred rarely. Paleolimnological analyses of chrysophyte scales from a sediment core from Bat Lake indicated that the lake's recent history was dominated by *Synura sphagnicola* scales, and the site was naturally acidic, although *Mallomonas hindonii* scales increased in relative abundance in the most recent sediments, indicating some additional acidification (J.P. Smol, unpublished data). Needless to say, naturally acidic and fishless lakes are not prime candidates for liming and restocking programs.

A second lake, which, according to paleolimnological analyses of diatoms (Dixit et al. 1992), was naturally acidic was Ruth-Roy Lake. Unfortunately, *Chaoborus* mandibles in the sediment core were very rare. Only a single mandible of *C. punctipennis* was recovered from the core. Therefore, no definitive statement can be made about change in the status of the fish populations. Ruth-Roy Lake is currently fishless (Polkinghorne and Gunn 1981) and was originally reported as fishless by Harvey (1975). The lack of *Chaoborus* remains in the sediments is not surprising, however, because a survey of the lake in 1982 failed to capture a single larva (Yan et al. 1985).

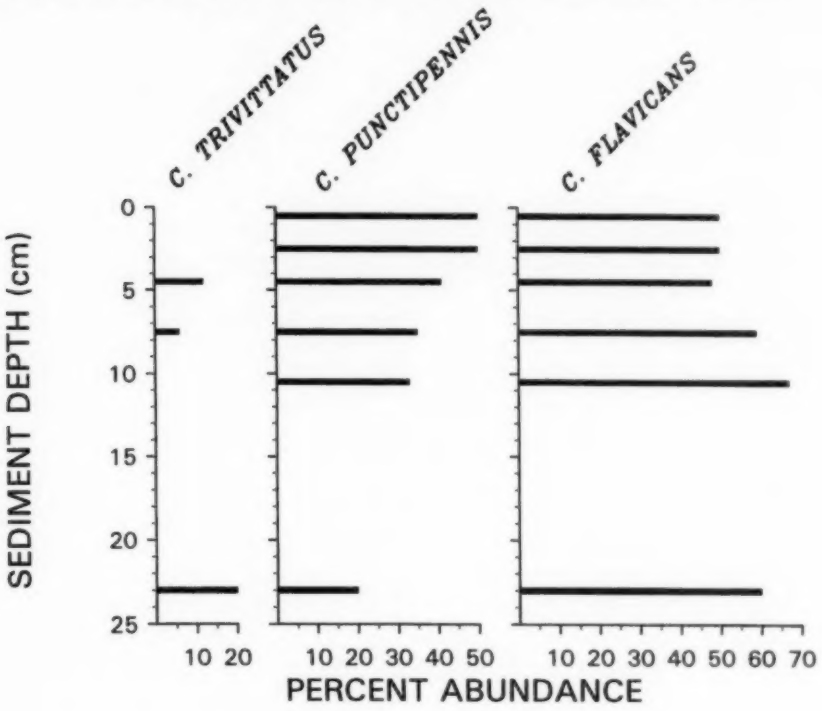
General discussion

These results reinforce the utility of *Chaoborus* remains as indicators of past fish populations as demonstrated in several earlier studies (Johnson et al. 1990; Uutala 1990; Kingston et al. 1992; Uutala et al. 1994; Lamontagne and Schindler 1994; Miskimmin and Schindler 1994). Cases such as Ruth-Roy and Clearwater lakes, where mandibles were too rare to make conclusions (both in the sediments and the plankton; see Yan et al. 1985), have thus far been rare. The only other such case in the aforementioned studies was one of the nine lakes studied by Johnson et al. (1990). Such cases are not a fault of the method, but are simply detection limit problems.

Information on past water quality and fisheries is important for effective planning of management practices. A lake, such as Bat Lake, that appears to be naturally fishless, would clearly not be a good candidate for remedial treatments and fish stocking. Whereas this is not particularly surprising because Bat Lake is a bog lake, similar cases have been found among drainage lakes (Uutala 1990).

The *Chaoborus* technique primarily assesses the presence or absence of planktivorous fishes. Thus, in the case of Crowley Lake, where it appears that fish have always populated the lake, detecting the addition of a new species may not be possible.

Fig. 4. *Chaoborus* profile from Crowley Lake. The number of mandibles per sample ranged from 15 to 20, except for the 10- to 11-cm sample, from which only 3 mandibles were recovered.



The cases of the acidified lakes show the usefulness of the technique. Swan Lake apparently lost its fish populations and would seem to be a good candidate for remedial action and stocking of appropriate fish species should its water chemistry eventually stabilize near its preacidification level.

Hannah Lake is particularly interesting in that *C. americanus* appeared in the core to indicate that planktivorous fish had disappeared. Similar to findings in Shoelace Lake in the Algonquin Park region (Uutala et al. 1994), *C. americanus* apparently declined following the successful recolonization of the lake by fish. Because the topmost sediments were not available for analysis in Middle Lake, we cannot determine if a similar decline occurred in this lake.

Interestingly, and not surprisingly, there is much variation in the tolerance of fish assemblages to adverse water chemistry. In this study, the inferred pH at the time of inferred fish losses in Middle, Hannah, and Swan lakes was 5.0 or below (Dixit et al. 1987, 1989*b*). This was also the inferred pH coincident with the complete loss of fish in Deep Lake in the Adirondack Mountains (Uutala 1990). Clearly, however, the survival and reproduction of individual species can begin to be affected at higher pH levels, such as reported by Schindler et al. (1985) and summarized by Baker (1990). Such adverse impacts can be serious enough that certain lakes have become devoid of fish even though average pH levels did not drop below about 5.5 (Uutala et al. 1994). On the other hand, some Sudbury region lakes still support fish despite having pH levels below 5.0 (Conlon et al. 1992).

These studies are important for water quality managers and fisheries managers who are considering rehabilitation of certain lakes. Clearly, restocking of fish is probably not desirable if the lake is naturally devoid of fish. Knowing that the lake once supported fish and what its past water chemistry was like, however, provides good targets for rehabilitation. For example, if the preimpact pH of the lake was 6.0, mitigation efforts should be controlled such that pH is not raised above this level.

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Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation

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Abstract: The minisatellite DNA probe Ssa1 was used to survey variation in 19 British Columbia, 1 Yukon, 1 Alaskan, and 1 Russian populations of coho salmon (*Oncorhynchus kisutch*). Hybridization of Ssa1 to DNA restricted with five restriction enzymes revealed 2–12 restriction fragments derived from a single locus. Geographically, although no marked regional definition was found within stocks from northern British Columbia, the lower mainland, the Fraser River, and Vancouver Island, genetic variation between individual stocks was high. Fish were correctly identified to stock of origin with an accuracy of 45% using discriminant analysis and a jackknifed classification procedure with a 22-stock baseline. Estimated mixture proportions for the contributing stock in test samples comprising a single stock averaged 100% using a fixed baseline of 22 stocks (i.e., no error), and 89% when the baselines were resampled. Further, the stock composition of simulated mixed fishery samples containing up to seven southern British Columbia stocks was estimated within an average of 1.4% of the actual mixing portions, on the basis of a resampled baseline.

Résumé : La sonde d'ADN minisatellite Ssa1 a été utilisée pour étudier les variations dans 19 populations de saumon coho (*Oncorhynchus kisutch*) de Colombie-Britannique, 1 du Yukon, 1 d'Alaska, et 1 de Russie. L'hybridation de la sonde Ssa1 à l'ADN après l'action de cinq enzymes de restriction a révélé 2–12 fragments de restriction provenant d'un seul locus. Bien qu'on n'ait pas observé de différences marquées à l'échelle régionale dans les populations du nord de la Colombie-Britannique, des basses terres du Fraser, du Fraser et de l'île de Vancouver, les variations génétiques entre les populations individuelles étaient grandes. On a correctement identifié la population d'origine des poissons avec une exactitude de 45% à l'aide de l'analyse discriminante et de la méthode de classification dite du « canif » à partir d'une base de 22 populations. La proportion estimée de la population constituante dans des échantillons comprenant une seule population était en moyenne de 100%, à partir d'une base fixe de 22 populations (c.-à-d. erreur nulle) et de 89% à partir de bases d'échantillonnage répétées. En outre, on a estimé que la composition d'échantillons simulés de captures de pêche mixte renfermant jusqu'à sept populations du sud de la Colombie-Britannique ne s'écartait pas en moyenne de plus de 1,4% de la composition d'un échantillon réel, à partir d'une base d'échantillonnage répétée.
[Traduit par la Rédaction]

Introduction

Accurate estimation of stock composition is important in the management of fisheries directed at Pacific salmon. A variety of techniques have been used to estimate stock composition in Pacific salmon, but for coho salmon (*Oncorhynchus kisutch*), coded wire tagging (Jefferts et al. 1963) has been the main method (Shaul and Clark 1990). Some variation at enzyme loci has been observed (Bartley et al. 1992; Wehrhahn and Powell 1987; Utter et al. 1973), but this variation has not been systematically applied to estimate stock composition. Of the five Pacific salmon species, coho salmon show the least amount of genetic variation at enzyme loci (Wehrhahn and Powell 1987). As a consequence, the use of electrophoretic data to distinguish regions or stocks of coho salmon in mixed population fishery samples has had only limited success (Milner 1993). However, a few studies showed small levels of genetic variation between regions at specific loci. Utter et al. (1973) showed that genetic variation exists in transferrin alleles between stocks from the Columbia River and the Washington coast that could be useful in the regional identification of coho salmon. In addition, Wehrhahn and Powell (1987) found frequency differences in Pgi-3 and Ldh-4 alleles between fish from the lower coastal mainland of British Columbia (B.C.) and Vancouver Island. However, although these studies suggest that some local regional variation exists, variation at the level of individual populations has not been found.

Because of the relatively low level of variation found at enzyme loci, we chose to examine variation at the DNA level using highly polymorphic variable number of tandem repeat

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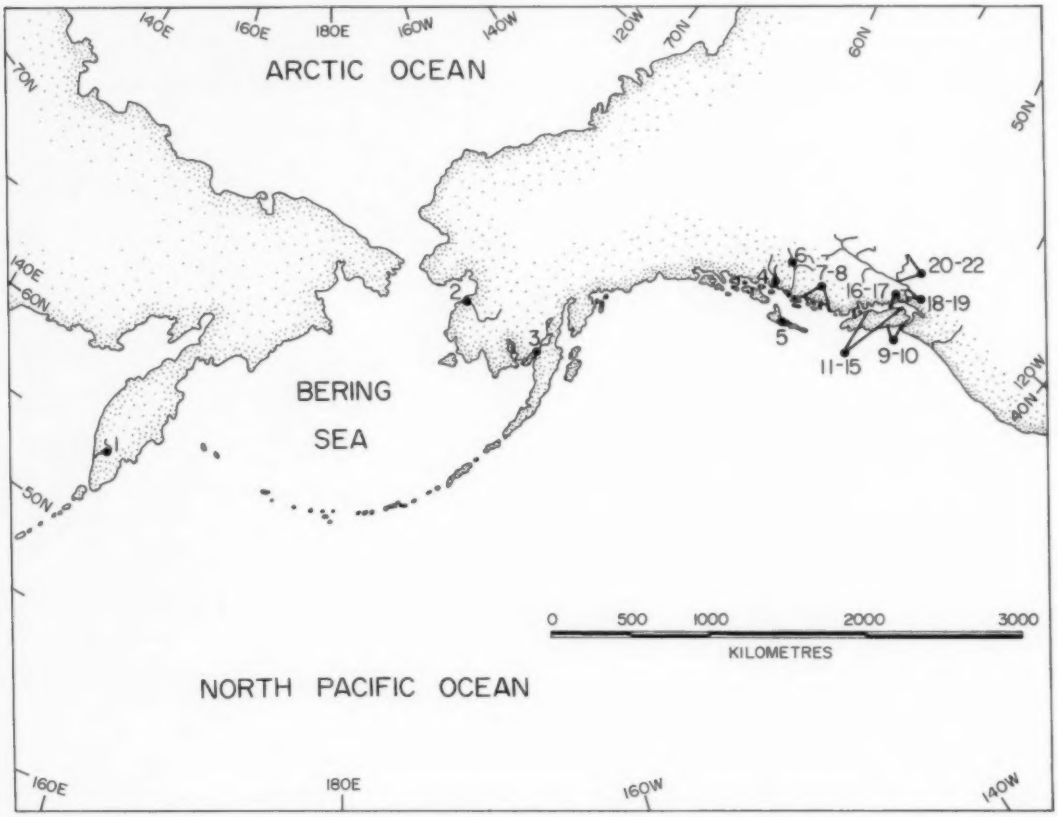
Fig. 1. Location of coho salmon stocks used in DNA analysis. Stocks include (1) west Kamchatka, Russia, (2) Yukon, (3) Bristol Bay, Alaska, (4) Stikine, (5) Pallant, (6) Toboggan, (7) Kitimat, (8) Atnarko, (9) Robertson, (10) Nitinat, (11) Quinsam, (12) Puntledge, (13) Big Qualicum, (14) Nanaimo, (15) Shawnigan, (16) Tenderfoot, (17) Capilano, (18) Chehalis, (19) Chilliwack, (20) Coldwater, (21) Salmon, and (22) Eagle.



(VNTR) loci. VNTR loci that contain tandemly repeated arrays of 10–75 nucleotides comprise a class of repetitive DNA in the nuclear genome called minisatellite DNA (Jarman and Wells 1989). The extreme polymorphism found at minisatellite loci results from allelic differences in the numbers of repeats (Jeffreys et al. 1985). One way to examine minisatellite loci for variation is through the use of minisatellite DNA probes hybridized to genomic DNA digested with restriction enzymes. When hybridized at low stringency, these minisatellite DNA probes often recognize multiple loci, leading to the complex banding patterns that have been referred to as DNA fingerprints (Jeffreys et al. 1985). We utilized a minisatellite DNA probe, Ssa1, derived from Atlantic salmon (*Salmo salar*) by Bentzen et al. (1993). Ssa1 hybridizes to one or two loci in salmon.

In this study, we examine nuclear DNA variation in populations of coho salmon at the Ssa1 minisatellite locus. The goal of the study was to determine the usefulness of minisatellite DNA in the identification of coho salmon to specific stocks or regions. The ability to identify individual fish to stock and the accuracy of estimated stock compositions are examined.

Materials and methods

DNA samples and extraction

Liver or blood samples were taken from approximately 50 spawning adult coho salmon at each of 22 locations in British Columbia, the Yukon, Alaska, and Russia during 1987–1992 (Fig. 1 and Table 1). Locations were chosen to represent a wide range of populations that act as discrete breeding units or stocks (according to definition by Ricker 1972), although the "stock" status of these populations has not yet been confirmed by estimations of rates of migration between populations. Samples were stored frozen at –20°C or stored in 95% ethanol at 4°C until DNA extraction. Prior to DNA extraction, ethanol was removed by centrifugation for ethanol-preserved samples. Approximately 0.3 g of liver tissue shaved from liver samples was placed in 15 mL of proteinase K lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 1% SDS, 0.2 μg/mL proteinase K) and incubated at 37°C overnight. Alternatively, 250 μL of blood (ethanol removed) was used. After overnight digestion, proteins were precipitated by the addition of 10 mL of 5 M NaCl,

mixed briefly, and centrifuged at 12 000 × *g* for 20 min. Supernatant was extracted once with phenol–chloroform (1:1) to further remove proteins and excess salt. DNA was precipitated in 2.5 volumes of 95% ethanol and incubated at –70°C for a period ranging from 1 h to overnight. High molecular weight DNA was removed from the ethanol with a glass rod and placed in Eppendorf tubes containing 70% ethanol. DNA was then pelleted by centrifugation, air dried at room temperature, and resuspended in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).

Southern blots and hybridization

Approximately 4.5 µg of genomic DNA from each fish was digested with each of five restriction enzymes (3 units/µg), *Hae*III, *Hin*fI, *Rsa*I, *Mbo*I, and *Alu*I. After a 3- to 5-h incubation at 37°C, DNA samples were loaded into 0.7% agarose gels. Each agarose gel contained 30 wells; 25 wells were loaded with genomic DNA, 1 of which was a control fish run on every gel, and 5 wells were loaded with lambda DNA digested with *Hin*dIII as a molecular weight standard. Most gels contained individuals from two to four populations, and each population was run on numerous gels. Each gel contained samples digested with the same restriction enzyme. DNA was size fractionated by electrophoresis of the 0.7% agarose gels run in TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) at 35 V for 16 h. Gels were then Southern blotted (Southern 1975) and UV cross-linked.

The minisatellite probe Ssa1 (Bentzen et al. 1993; Bentzen and Wright 1993) was used to assay variation at VNTR loci of coho salmon. The Ssa1 probe is comprised of a number of 16 base pair repeats and detects variation in the number of tandemly arranged copies of the repeat at a single locus in coho salmon (see family analysis).

Ssa1 was labelled with [^{32}P]dATP using random primer extensions (Feinberg and Vogelstein 1983). Membranes were placed in rotisserie hybridization oven bottles (4–6 membranes per bottle; Robbins Scientific) and prehybridized overnight at 65°C in 20 mL of prehybridization solution (7.0% SDS, 1.0 mM Na_2EDTA (pH 8.0), 1.0% bovine serum albumin, 0.263 M Na_2HPO_4). Hybridization was followed by the addition of new hybridization solution, the denatured Ssa1 probe, and 10 µg of denatured sheared calf thymus DNA, followed by another overnight incubation at 65°C. After hybridization, membranes were washed twice for 15 min at room temperature in 2× SSC (300 mM NaCl, 30 mM trisodium citrate, 0.1% SDS, once for 30 min at 65°C in 1× SSC, 0.1% SDS, and once for 15 min at room temperature in 1× SSC. Membranes were then exposed to X-ray film (Kodak XOMAT-AR) for 1–3 days at –70°C.

DNA blot analysis and binning

Autoradiographs were scanned using a Kodak high-resolution two-dimensional charge coupled device (CCD) camera with a 512 × 512 pixel density. Bioimage software (Millipore Corp. Imaging Systems, Ann Arbor, Mich.) was used to analyze the blots. The lambda size markers, which were present in five lanes across the blots, were used to form a size grid across the blots upon which bands between 2 and 23 kilobase pairs (kbp) in size were measured. Within this range, Ssa1 resolved between 2 and 12 polymorphic bands with each of the five restriction enzymes used. In addition, there were four to six bands below 2.0 kbp in size that were not scored because of variation in their resolution between blots. Because of the nearly continuous distribution of molecular weights sampled, and the large number of individuals and populations examined on multiple gels, an estimate of band size measurement error was made following Galbraith et al. (1991). Control fish that contained bands with sizes that spanned the 2- to 23-kbp range in size were run on each blot, with a different control fish for each enzyme. A total of 41 different molecular weight (MW) bands from all control fish combined were used to calculate the mean and standard deviation (SD) of MW (in kilobase pairs) over all gels. A linear regression of the natural logarithm of the standard deviation of MW (σ_{MW}) on mean MW (μ_{MW}) was used to interpolate standard deviations for bands with different molecular weights between 2 and 23 kbp. The regression equation was

$$\ln\sigma_{MW} = 0.151\mu_{MW} - 3.96$$
$$r^2 = 0.80,\ P < 0.001,\ N = 41$$

The regression equation was used to establish molecular weight size intervals, or bins, that were 5 SD wide, over the 2- to 23-kbp size range in which bands were measured. A total of 41 continuous bins was established by assuming that a bin width of 5 SD (+2.5) would account for approximately 99% of the measurement error according to the gel set-up employed in this study (Gill et al. 1990; Galbraith et al. 1991). Bins widths increased with molecular weight, ranging from 0.2 kbp in the 2.0- to 2.2-kbp bin size interval to 3 kbp in the 20- to 23-kbp bin size interval, hence the positive correlation between μ_{MW} and $\ln\sigma_{MW}$.

Table 1. Stocks analyzed for variation at VNTR loci recognized by the minisatellite probe Ssa1.

Stock		Region	*N*
Atnarko	(At)	Northern B.C.	69
Kitimat	(Ki)	Northern B.C.	69
Pallant	(Pa)	Northern B.C.	83
Toboggan	(To)	Northern B.C.	18
Stikine	(St)	Northern B.C.	6
Robertson	(Ro)	WCVI	71
Nitinat	(Ni)	WCVI	42
Quinsam	(Qu)	ECVI	98
Puntledge	(Pu)	ECVI	40
Big Qualicum	(BQ)	ECVI	73
Nanaimo	(Na)	ECVI	24
Shawnigan	(Sh)	ECVI	37
Tenderfoot	(Te)	Lower mainland	53
Capilano	(Ca)	Lower mainland	35
Coldwater	(Co)	Upper Fraser	29
Salmon	(Sa)	Upper Fraser	17
Eagle	(Ea)	Upper Fraser	45
Chehalis	(Che)	Lower Fraser	30
Chilliwack	(Chi)	Lower Fraser	69
Bristol Bay	(Br)	North of B.C.	21
Yukon	(Yu)	North of B.C.	17
West Kamchatka	(Ka)	Russia	29

Note: The regional location of each of the stocks and numbers of individuals are shown as well as the stock abbreviations in parentheses. WCVI, west coast of Vancouver Island; ECVI, east coast of Vancouver Island. *N* is the number of individuals sampled in each stock.

Bands from individual fish digested with each enzyme were binned into each of the 41 molecular weight size intervals. Each fish produced 2–12 bands, and consequently most of the bins contained zero or one band, and none contained more than two bands. Because of the measurement error and the contiguous molecular weight size intervals used, it was possible for homologous bands in different individuals to fall into more than one bin when the MW was close to the class limits of the bins. However, Gill et al. (1990) showed that this binning procedure did not differ significantly from that using a sliding window routine, which bases bin intervals on band frequency data. The sliding window routine was not possible with the present data set because the relatively large number of restriction fragments and extreme polymorphism detected by SsaI resulted in a nearly continuous distribution of molecular weights. Hence, nearly all of the 41 bins contained a band from at least one specimen for each of the five restriction enzymes examined.

Statistical analysis

Family data

A pedigree study was conducted to establish the Mendelian inheritance of minisatellite bands and the number of loci recognized by SsaI. Three male and three female coho salmon from a domesticated strain of Kitimat River origin were crossed to produce three full-sib families. Sixteen to 22 offspring from each family were analyzed using *Mbo*I, *Hin*fI, *Alu*I, and *Rsa*I restriction enzymes. The use of four enzymes was sufficient to establish the Mendelian inheritance of SsaI and the number of loci recognized by SsaI; thus, *Hae*III was not included in the analysis. Mendelian inheritance was analyzed using the χ^2 statistic.

Interannual data

Interannual variation of binned data was examined for six stocks with individuals collected over 2–4 years each, including Kitimat (1987, 1988, 1991, 1992), Big Qualicum (1988, 1992), Quinsam (1987, 1988, 1992), Pallant (1987, 1992), Robertson Creek (1988, 1992), and Chilliwack (1988, 1992). The χ^2 test accompanied by Monte Carlo simulations of the distribution of the χ^2 statistic was used to estimate the probability of obtaining a value equal to or greater than that observed (Roff and Bentzen 1989). One hundred to 1000 randomizations of the original data were used in the program MONTE of the REAP software package (McElroy et al. 1992). Restriction enzymes, each with 41 bins of input data, were analyzed separately.

Because of the large number of tests made (6 stocks × 5 enzymes = 30 tests), significance levels were adjusted for the number of tests carried out using the method of Hochberg (1988), as suggested by Lessios (1992). In this method, tests are ordered by decreasing probability value. If the highest probability is <0.05, all tests are considered significant. However, if the highest probability is >0.05, then comparisons of subsequent probabilities continue, but the significance level is adjusted by division by the number of tests performed plus one. When a test is significant at the adjusted significance level, testing stops, and it and all subsequent tests are deemed significant.

Interstock data

Interstock variation in binned data was statistically analyzed for (*i*) the significant contributions of individual bins to interstock variation, (*ii*) the general distances between stocks to assess geographic trends in variation, (*iii*) the ability to identify individual fish to stock, and (*iv*) the ability to estimate stock composition of mixtures. The contribution of individual bins to significant variations between stocks was examined using the *G* test statistic in SAS (SAS Institute Inc. 1989) for each of the 41 bins present in each of the five restriction enzyme data sets. For the rest of the statistical procedures, the five restriction enzyme data sets were combined into a master data set containing 22 populations and 205 bins. Thus, for each individual fish sampled (975 in all), the data set consisted of 205 variables (bins) with a value of 0, 1, or 2 in each (representing the number of bands in each bin). A generalized squared distance (D^2; Pimentel 1979) was calculated from mean bin counts over all 205 bins for each pair of populations. The matrix of distances was then clustered using the neighbor-joining algorithm (NEIGHBOR in PHYLIP; Saitou and Nei 1987; Felsenstein 1990) and a dendogram was developed.

The ability to classify individual fish into stocks was examined using linear discriminant analysis with the DISCRIM procedure in SAS (Pimentel 1979). The level of accuracy in assigning individual fish to their stock of origin was assessed using the jackknife procedure.

The ability to estimate stock composition from samples taken from a mixed stock fishery was examined using a maximum likelihood model (Fournier et al. 1984). The maximum likelihood model uses probability distributions for each of a number of independent characters to define the probability of choosing a fish with a particular set of characteristics. Principal component analysis (PRINCOMP procedure in SAS on correlation matrix) was used to incorporate the data from all five enzymes, into 205 uncorrelated variables (principal components) that accounted for 100% of the observed variation. Each principal component is a linear combination of the original 205 variables, which have been standardized by subtracting the corresponding overall mean and dividing by the corresponding standard deviation over all stocks. The input to the maximum likelihood model required discrete frequency counts for each variable, and as outlined by Beacham et al. (1995), the continuous distributions of the principal component scores were represented as 12-bin histograms. Each stock was then characterized as an input matrix of 205 rows (principal components) and 12 columns (counts of principal component scores in each of the 12 bins).

The accuracy of the maximum likelihood model in the estimation of stock compositions of coho salmon was examined with both fixed and resampled (bootstrapped) baselines containing all 22 stocks. Fixed baseline data sets assume that the baseline data represent the stocks perfectly. More realistically, resampled baseline data sets emulate the randomness involved in the collection of baseline data under the assumption that the baseline samples are representative, random samples of the stocks. Single stock simulated fishery (test) samples of 50 fish were generated randomly with replacement, and 25 different single stock mixtures were analyzed with both fixed and resampled baseline data sets, with the mean and standard deviation of the estimated stock composition determined. In addition, stock composition was estimated for simulated

Table 2. Restriction fragment sizes (kb) for alleles present in three family crosses of coho salmon when analyzed with four restriction enzymes.

Allele	*Mbo*I	*Hin*fI	*Alu*I	*Rsa*I
A	2.21+6.05+12.69	4.99+14.55	2.84+13.09	2.40+7.80+15.88
B	2.21+6.05+12.69	4.57+14.55	2.84+13.09	2.40+7.80+15.88
C	3.93+5.16+5.88+9.04+13.88	<2.0+2.09+3.15+22.51	2.84+22.56	2.35+3.23+3.67+7.57+>23.1
D	2.38+3.67+5.77+6.25+11.77	2.33+4.53+8.33+10.87+15.66	3.56+6.01+12.69	2.08+3.74+6.07+6.80+16.97
E	7.70	2.10+2.33+2.43+4.90	3.49	3.45
F	5.27+5.75+6.18	<2.0+5.74+>23.1	3.36+3.62+>23.1	2.35+3.50+6.07+8.10+>23.1
G	9.21+20.96	2.74+3.52+4.46+4.67+5.10+6.55	2.47+3.52+4.95+11.25	2.80+6.60+9.10+14.00

Table 3. Genotypes of three families of coho salmon used in the pedigree analysis.

Parental genotypes	Progeny genotypes
M1F1	
Male 1 (CC) × female 1 (AB)*	9 AC, 9 BC
Male 1 (CC) × female 1 (AA)	18 AC
M3F5	
Male 3 (AE) × female 5 (CD)	3 AC, 3 AD, 4 CE, 6 DE
M4F2	
Male 4 (AC) × female 2 (FG)	4 AF, 5AG, 6 CF, 4 CG

*Only *Hin*fI resolved the B allele.

multistock fishery samples containing stocks from specific regions, such as the Strait of Georgia, northern British Columbia, and the Fraser River.

Results

Banding patterns of Ssa1

In a given individual, the number of bands between 2 and 23 kbp in size varies from 2 to 12 with different restriction enzymes (Fig. 2). Further, there are a variable number of bands, from 4 to 6, present that are less than 2.0 kbp in size that were not scored in the present study because of variation in their resolution between blots.

Family analysis

Pedigree analysis of three coho families revealed that the Ssa1 probe detected a single highly polymorphic locus with diploid inheritance. Each allele consisted of between one and six restriction fragments (Table 2). The multiple fragments constituting a single allele displayed complete linkage (i.e., complete cotransmission, or lack of cotransmission, to progeny) and Mendelian inheritance (transmission to 50% of progeny from homozygous parents) (Table 3). For instance, in the cross between male 3 and female 5, analysis of the inheritance of banding patterns between parents and offspring revealed that four alleles were resolved by *Hin*fI (Fig. 3). The female contributed allele C, with five bands (<2.0, 2.09, 3.15, and 22.51 kbp in size; Table 2), and allele D, with five bands (2.33, 4.53, 8.33, 10.87, and 15.66 kbp in size). Seven offspring out of 16 carried the C allele, while the remaining 9 carried the D allele (Table 3). In this example, maternal and paternal alleles shared two fragments of the same size, 2.33 kbp (male E allele and female D allele) and 2.09–2.10 kbp (male E allele and female C allele), and one fragment of a similar size (visually), 14.55 kbp (male A allele) and 15.66 kbp (female D allele).

Fig. 2. Portions of autoradiographs of coho salmon DNA restricted with *Mbo*I (A), *Rsa*I (B), or *Hin*fI (C) and hybridized with Ssa1. Portions of each autoradiograph were included to illustrate variation observed. Molecular weights are given in kilo base pairs.

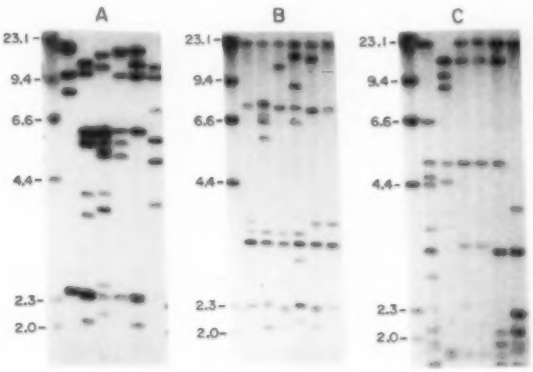
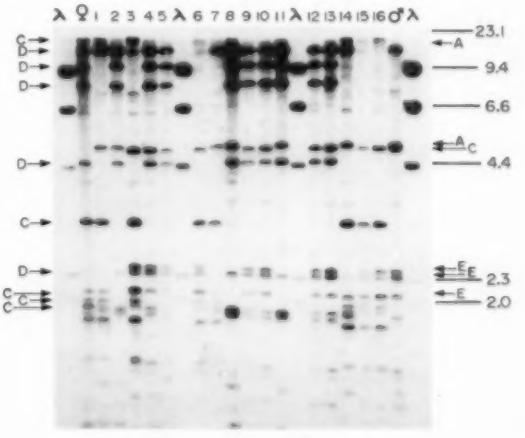


Fig. 3. Audoradiograph of the male-3 female-5 family restricted with *Hin*fI. Restriction fragments comprising individual alleles are marked for parents (see Table 2 for sizes) and offspring and numbered. Molecular weights are given in kilo base pairs.



Seven alleles ranging in minimum size from 3.45 to 42 kbp (calculated by the sum of band sizes for each allele) over four restriction enzymes were identified among the six parents (Table 3). The estimates of allele size and the number of restriction sites present in each allele are conservative because only DNA fragments between 2 and 23 kbp could be scored reliably from our autoradiographs. For all four restriction enzymes with 4-bp recognition sequences, all seven alleles surveyed at the Ssa1 locus were detectable in this study in that they possessed one or more restriction fragments between 2.0 and 23.0 kbp.

For each restriction enzyme, some Ssa1 alleles shared DNA fragments of the same or very similar molecular weights. However, the pattern of shared fragments among alleles differed by enzyme. For example, restriction with *Alu*I revealed a 2.84-kbp DNA fragment in alleles A, B, and C. Similarly, DNA fragments between 4.90 and 5.10 kbp were present in alleles A, E, and G for *Hin*fI, and DNA fragments between 4.46 and 4.57 kbp were present in alleles B, G, and D for *Rsa*I. In the restriction fragment patterns of these two enzymes, the absence of 0.4 kbp in allele B relative to allele A was obvious because it occurred in moderately sized restriction fragments (4.5 and 7.4 kbp, respectively) (Tables 2, 3). In the banding patterns from other restriction enzymes, the absence of the 0.4-kbp sequence from allele B probably was not detected because of its occurrence on a restriction fragment less than 2.0 kbp in size (and therefore not scored) or on a restriction fragment greater than 12.0 kbp, at which size the difference in size would not be resolved on the gel.

Interannual variation

Interannual variation was detected in five out of six stocks, including Chilliwack, Kitimat, Pallant, Quinsam, and Robertson Creek. Out of 30 tests performed, 10 showed significant variation at the $P < 0.05$ level (adjusted for the number of tests). Variation was only detected by a single restriction enzyme for Big Qualicum, Chilliwack, and Kitimat stocks, and by two to three restriction enzymes for Pallant, Quinsam, and Robertson Creek stocks.

To compare the degree of interannual variation with interstock variation, we conducted Monte Carlo simulations of the χ^2 test on two-stock comparisons between stocks with more than one year-class (combined for the comparison) and stocks located in close geographic proximity. Interstock comparisons included Kitimat and Atnarko, Big Qualicum and Quinsam, Chilliwack and Chehalis, Pallant and Kitimat, and Robertson Creek and Nitinat. All of the enzymes showed significant variation between adjacent stocks at the $P < 0.05$ level (25/25 tests). The detection of significant interstock variation in each interstock comparison over all enzymes suggests that interstock variation was greater than interannual variation within stocks.

Interstock variation

Bin variation

Because the data set is so large (205 bins by 975 fish in 22 populations), bin counts are not shown for all enzymes. Instead, we included bin counts for one enzyme, *Mbo*I, in the Appendix and selected variable bins over all enzymes in Fig. 4. Variation in bin frequencies among stocks was apparent, and in many instances there were regional patterns (Fig. 4). On average, 26 of the 41 bins showed significant interstock variation for each of the five restriction enzyme data sets (G test, $P < 0.05$, range of 22–30 significant bins per enzyme). In addition, all 41 bins within each of the five restriction enzyme data sets contained bands in at least one stock (e.g., *Mbo*I, Appendix).

Enzyme variation

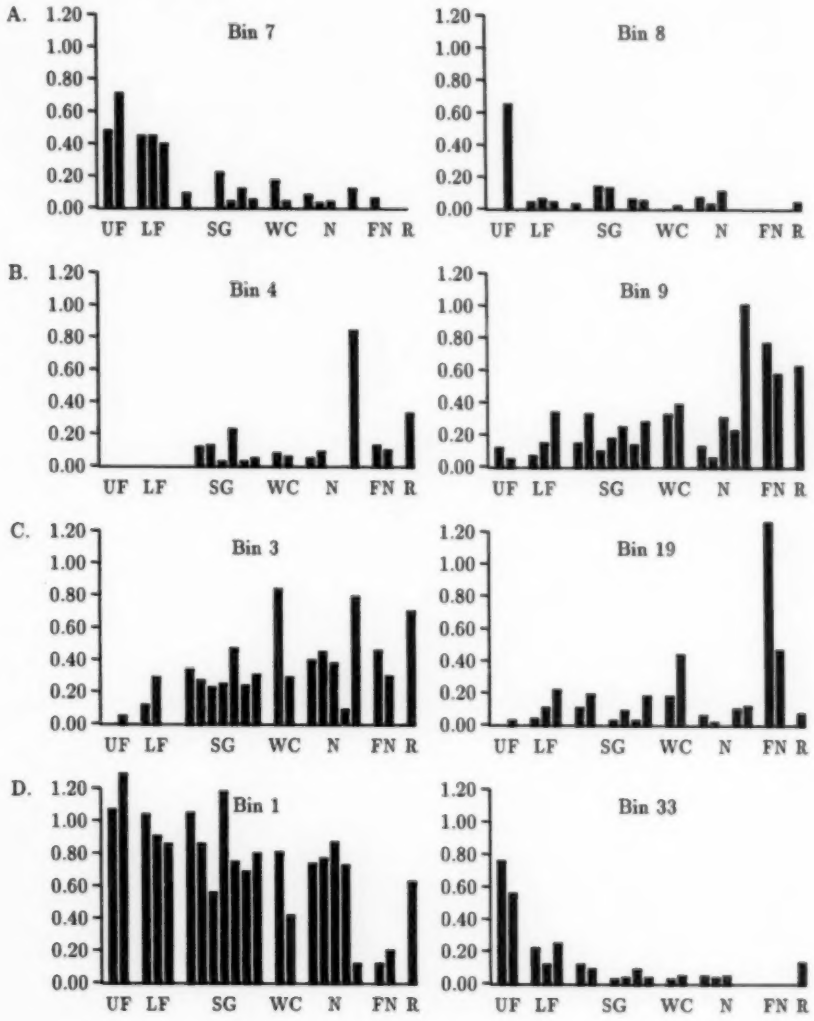
Variation in band size at a minisatellite locus is usually due to variation in the numbers of repeats between restriction sites, not in restriction site differences. Therefore, banding patterns should not vary considerably between restriction enzymes, and as a result, all of the variation at a minisatellite locus should be sampled by a single restriction enzyme. Results in the present study, however, indicated that the discrimination of specific stocks varied among restriction enzymes, and that the use of multiple restriction enzymes increased the accuracy of stock discrimination. The contribution of the individual enzyme data sets was examined using linear discriminant analysis run on each restriction enzyme data set independently, and then on sets of two, three, four, and finally all five enzymes together. Classification accuracy, characterized as the mean percentage correct over all stocks, increased with the number of restriction enzymes used in the discriminant function (Fig. 5). Percentage correct for jackknifed individuals, i.e., individuals not used in the formulation of the discriminant function, shows how well discriminant analysis is able to predict stock of origin of fish it has not seen before. Percentage correct of jackknifed test fish increased with increasing numbers of restriction enzymes used in the analysis, from a mean of 27% with one enzyme to 42% with all five enzymes. Given 20 possible stocks, random classification (i.e., no discrimination) would yield a classification accuracy of only 5%.

A comparison of percentage correct for each stock using each individual restriction enzyme showed that the enzymes varied in their discrimination of specific stocks. For instance, *Hae*III showed no discrimination of the Yukon stock, with a percentage correct of 6%, whereas *Hin*fI discriminated this stock for 67% of individuals. Therefore, single restriction enzymes did not sample all of the interstock variation present at the Ssa1 locus, and the use of multiple restriction enzymes was essential for accurate discrimination between stocks.

Identification of individuals to stock

We analyzed 975 fish from 22 stocks, with 6 (Stikine) to 98 (Quinsam) individuals per stock (Table 1). The classification accuracy of jackknifed test fish ranged from 0% at Stikine to 82.4% at Salmon River, with a mean of 44.6% correct (Table 4). Variation between stocks in the accuracy of classification may result from a less than complete sampling of within-stock variation. If stocks are similar in their levels of within-stock variation, stocks with low numbers of individuals sampled should show the lowest classification accuracies. For example, for the Stikine stock, in which only six fish were sampled, no fish was correctly identified. However, classification accuracy was not always lower for stocks with small numbers of fish. For example, the Salmon River stock was represented by only 17 fish, and 82.4% of fish were correctly identified, while at Puntledge, with 40 fish sampled, only 25% of fish were correctly identified. Therefore, stocks may differ

Fig. 4. Mean numbers of bands in selected bins (containing variability among stocks) for 22 stocks of coho salmon with the regional groups abbreviated as follows: UF, upper Fraser; LF, lower Fraser; SG, Strait of Georgia; WC, west coast of Vancouver Island; N, north (within B.C.); FN, far north (north of B.C.); and R, Russia. See Table 1 for stocks contained in each of these regions. Restriction enzymes used were *Mbo*I (A), *Rsa*I (B), *Hin*fI (C), and *Hae*III (D). Limits for the bin intervals were 1944–2100 bp (1), 2262–2426 bp (3), 2427–2595 bp (4), 2946–3128 bp (7), 3129–3315 bp (8), 3316–3508 bp (9), 5560–5832 bp (19), and 11 066 – 11 708 bp (33).



in the amount of within-stock variation at the Ssa1 locus, and as a result the numbers of fish necessary to sample all of this variation may also differ among stocks. In addition, some stocks may have higher levels of straying than others, and thus be less distinct.

Regional definition of stocks

To examine geographic trends in minisatellite variation along the coast, we calculated pairwise generalized squared genetic distances (D^2). These values ranged from 12.8 (Tenderfoot versus Kitimat) to 165.4 (Salmon River versus Stikine). Despite large D^2 values between stocks, few regional clusters of stocks were established by the neighbor-joining algorithm (Fig. 6). Three of the five Fraser River stocks clustered together, with a subcluster for the upper Fraser River stocks at Salmon and Eagle rivers. However, there was little, if any, regional definition between stocks from the Strait of Georgia, including stocks from the east and west coast of Vancouver Island and from the lower mainland, and stocks from northern British Columbia. Other stocks, such as Bristol Bay, Stikine,

Fig. 5. Mean percentage of individuals correctly identified to stock from linear discriminant analysis on the basis of the number of enzymes used in the analysis. Error bar equals two standard errors.

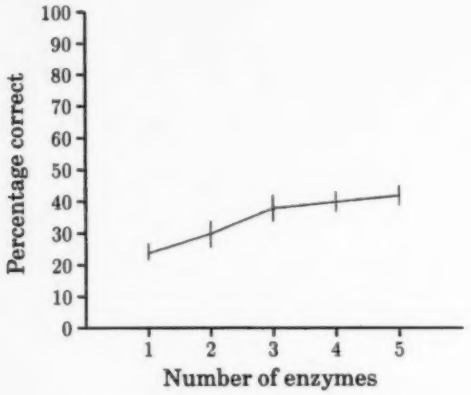


Fig. 6. Neighbor-joining dendrogram of relationships among 22 coho salmon stocks based upon Mahalanobis distances calculated from bin frequency data at the Ssa1 locus.

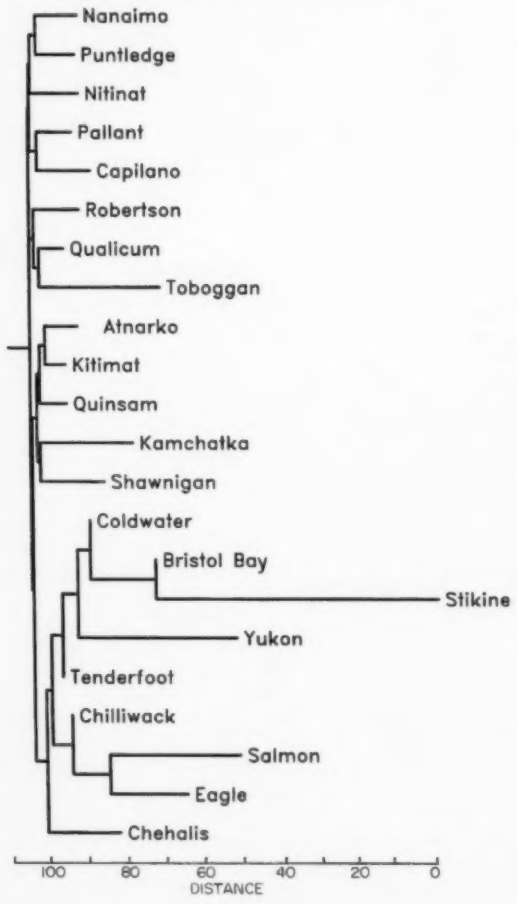


Table 4. Percentage of individuals correctly identified to stock by linear discriminant analysis of unknown test fish (i.e., jackknifed individuals).

Stock	Unknown	*N*
Atnarko	50.7	69
Kitimat	40.6	69
Pallant	67.5	83
Toboggan	44.4	18
Stikine	0	6
Robertson	62.0	71
Nitinat	52.4	42
Quinsam	49.0	98
Puntledge	25.0	40
Big Qualicum	52.1	73
Nanaimo	25.0	24
Shawnigan	56.5	37
Tenderfoot	37.7	53
Capilano	42.9	35
Coldwater	34.5	29
Salmon	82.4	17
Eagle	66.7	45
Chehalis	30.0	30
Chilliwack	42.0	69
Bristol Bay	28.6	21
Yukon	52.9	17
West Kamchatka	44.9	29
Overall mean	44.9	

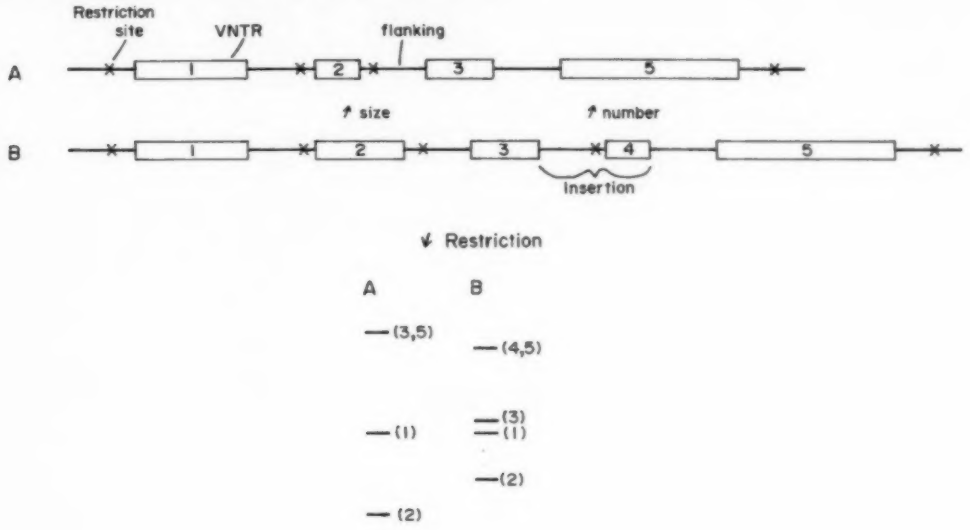
and Yukon, formed a distinct cluster, along with one upper Fraser stock, Coldwater. The most distinct stock was the Stikine, which had a sample size of only six fish. Although Stikine clustered with non-British Columbia stocks, the genetic distance between Stikine and all other stocks was, on average, greater than twice that found between other pairs of stocks.

Estimation of stock compositions

The utility of minisatellite polymorphisms for stock identification was evaluated using the maximum likelihood method analysis in several ways. Initially, test samples of 50 fish were simulated that contained only one stock and their composition was estimated with fixed and resampled baselines containing all 22 stocks. Using the fixed baseline, estimated stock compositions of the single-stock 'mixtures' averaged 100% for the 22 stocks evaluated. Results of the resampled baseline are shown in a matrix in Table 5, where the numbers on the diagonal show the estimated percentage composition of the tested stocks (with the mixture actually containing 100% of that stock) and numbers off the diagonal show which stocks the analysis erroneously estimated were present. Estimated proportions for the single contributing stock averaged 89.4% using the resampled baseline.

Simulated fishery samples containing 50-fish mixtures were also tested using the 22-stock fixed and resampled baselines. Stock composition was estimated with almost 100% accuracy using fixed baseline data, with deviations from correct percentages of each stock averaging 0.36% for Strait of Georgia stocks and 0.04% for the regional stocks (Tables 6, 7).

Fig. 7. Hypothetical schematic of the Ssa1 locus in coho salmon and the generation of polymorphisms. Two hypothetical alleles are shown. The A allele contains four numbered VNTR (minisatellite) sequences (1, 2, 3, and 5; box), each surrounded by flanking sequence (line), with four of the five flanking sequences containing restriction sites (X). The B allele differs from the A allele by an increase in size of VNTR-2, and an insertion of VNTR-4, along with an accompanying flanking sequence with an additional restriction site. Postulated restriction patterns of each of the alleles are illustrated below. In this example, although the two alleles share three of the VNTRs and there is no change in restriction sites within the common flanking sequences, the two alleles only share one restriction fragment. Thus, banding patterns can vary dramatically between alleles by mutation causing variation in the number and size of VNTRs within the Ssa1 locus.



Resampled baseline stock compositions were predicted with slightly less accuracy, with mean deviations from correct percentages of each stock of 1.66% for Strait of Georgia stocks and 2.44% for regional stocks.

We evaluated the reliability of estimates of stock composition in a more realistic multistock fishery. Simulated mixtures of 200 fish containing only southern British Columbia stocks (14 stocks in all) were resolved with fixed and resampled baselines. Accuracy was extremely high, with the estimated error averaging 0.13% (fixed baseline) and 1.4% (resampled baseline) of the actual percentages for each stock present in the mixture (Table 8). In addition, of the noncontributing stocks, only two were incorrectly estimated as present in the mixtures: Tenderfoot, at 0.03–0.07% (fixed baseline), and Robertson Creek, at 0.06% (resampled baseline).

Finally, the estimation accuracy of the five Fraser River stocks alone was analyzed. Single-stock 50-fish test samples were simulated for each of the five Fraser River stocks and evaluated with fixed and resampled baselines containing only the five Fraser River stocks (Table 9). Estimated stock compositions averaged 100% for the fixed baseline and 99.4% for the resampled baseline.

Discussion

Nature of polymorphism at the Ssa1 minisatellite locus

The fact that the multiple DNA bands detected by the Ssa1 minisatellite probe in coho salmon represent the fragmented alleles of a single locus is consistent with the fact that Ssa1 has been shown to hybridize strongly to a single polymorphic locus in each of Atlantic salmon (*Salmo salar*), steelhead trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarki*), and sockeye salmon (*Oncorhynchus nerka*), and to two linked loci in chum salmon (*Oncorhynchus keta*) (Bentzen et al. 1993; Taylor et al. 1994; Taylor et al. 1996). Allelic variation at minisatellite loci generally results from differing numbers of the repeat unit contained between a conserved pair of restriction sites located in the nonrepetitive flanking DNA (Jeffreys et al. 1988), although variation in the sequence of the repeat unit can also occur (Jeffreys et al. 1990). Thus, individual minisatellite alleles are commonly visualized as single restriction fragments of variable length or sequence defined by the conserved pair of external restriction sites. However, the Ssa1 locus of coho salmon, which is visualized as multiple restriction fragments of variable length and size, apparently has a different structure. It is postulated that nonrepetitive flanking sequences containing restriction sites are interspersed with regions of the Ssa1 minisatellite (VNTR) repeat (Fig. 7). The mechanisms behind the generation of polymorphism in Ssa1 are likely similar to that of normal (single) minisatellites, and include gene conversion (Jeffreys et al. 1993) and replication slippage (Wolf et al. 1991).

Coho salmon Ssa1 alleles contained up to six detectable restriction sites for a given enzyme and ranged in size up to at least 42 kbp. The fact that a probe specific for a 49-bp flanking region of Ssa1 in Atlantic salmon also hybridizes strongly to multiple fragments in restricted coho salmon DNA (Bentzen

Table 5. Estimated percentage compositions of simulated pure stocks of coho salmon.

	At	Ki	Pa	To	Ro	Ni	Qu	Pu	BQ	Na	Sh
At	88.4 (5.8)	0.8 (1.6)	0.8 (1.5)	0.0 (0.0)	8.5 (6.2)	0.0 (0.0)	0.2 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Ki	1.1 (2.0)	89.6 (5.2)	1.2 (2.0)	0.0 (0.0)	0.4 (1.5)	0.0 (0.0)	5.6 (5.3)	0.0 (0.0)	1.3 (2.1)	0.0 (0.0)	0.0 (0.0)
Pa	0.7 (1.4)	0.3 (0.8)	89.5 (5.5)	0.0 (0.0)	0.6 (1.5)	0.0 (0.0)	7.4 (4.1)	0.0 (0.0)	1.3 (0.0)	0.0 (0.0)	0.0 (0.0)
To	0.0 (0.0)	1.0 (3.8)	1.3 (3.5)	91.0 (7.5)	0.0 (0.0)	0.0 (0.0)	3.4 (5.3)	0.0 (0.0)	2.4 (4.4)	0.0 (0.0)	0.0 (0.0)
Ro	0.7 (1.4)	0.0 (0.0)	1.9 (3.4)	0.0 (0.0)	88.8 (6.2)	0.0 (0.0)	8.2 (5.7)	0.0 (0.0)	0.3 (0.8)	0.0 (0.0)	0.0 (0.0)
Ni	1.2 (2.3)	1.1 (2.1)	0.6 (2.1)	0.0 (0.0)	1.3 (2.7)	83.5 (8.7)	9.8 (8.3)	0.0 (0.0)	1.5 (3.0)	0.0 (0.0)	0.0 (0.0)
Qu	0.3 (1.0)	0.3 (1.2)	0.8 (1.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	97.7 (3.0)	0.0 (0.0)	0.6 (2.0)	0.0 (0.0)	0.0 (0.0)
Pu	0.2 (0.8)	0.1 (0.6)	0.3 (1.0)	0.0 (0.0)	1.1 (2.2)	0.0 (0.0)	5.2 (5.0)	90.6 (6.5)	1.2 (2.4)	0.0 (0.0)	0.0 (0.0)
BQ	0.0 (0.0)	0.0 (0.0)	8.6 (4.6)	0.0 (0.0)	1.0 (1.9)	0.0 (0.0)	6.6 (5.0)	0.0 (0.0)	88.4 (6.8)	0.0 (0.0)	0.0 (0.0)
Na	1.2 (3.6)	0.4 (2.0)	1.8 (3.1)	0.0 (0.0)	0.4 (1.3)	0.0 (0.0)	11.5 (9.2)	0.0 (0.0)	1.1 (2.9)	80.7 (11.5)	0.0 (0.0)
Sh	0.0 (0.0)	0.5 (1.3)	0.4 (1.4)	0.0 (0.0)	0.7 (2.3)	0.0 (0.0)	11.3 (6.4)	0.0 (0.0)	1.8 (3.9)	0.0 (0.0)	84.8 (7.9)
Te	2.1 (2.9)	0.8 (1.6)	3.4 (4.5)	0.0 (0.0)	1.8 (3.9)	0.0 (0.0)	8.3 (5.2)	0.0 (0.0)	1.9 (3.2)	0.0 (0.0)	0.0 (0.0)
Ca	0.2 (0.7)	0.0 (0.0)	2.4 (4.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.4 (5.6)	0.0 (0.0)	0.4 (2.1)	0.0 (0.0)	0.0 (0.0)
Co	0.0 (0.0)	0.9 (2.5)	0.4 (1.9)	0.0 (0.0)	0.9 (1.9)	0.0 (0.0)	5.0 (5.8)	0.0 (0.0)	0.4 (1.3)	0.0 (0.0)	0.0 (0.0)
Sa	1.2 (3.4)	0.0 (0.0)	0.7 (2.5)	0.0 (0.0)	0.4 (1.9)	0.0 (0.0)	10.7 (11.6)	0.0 (0.0)	0.3 (1.3)	0.0 (0.0)	0.0 (0.0)
Ea	0.3 (1.1)	0.4 (1.2)	0.4 (1.2)	0.0 (0.0)	1.4 (3.6)	0.0 (0.0)	7.1 (6.0)	0.0 (0.0)	1.3 (2.9)	0.0 (0.0)	0.0 (0.0)
Che	0.6 (3.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (1.3)	0.0 (0.0)	4.1 (5.7)	0.0 (0.0)	0.1 (0.6)	0.0 (0.0)	0.0 (0.0)
Chi	0.2 (0.7)	0.8 (2.3)	2.0 (2.5)	0.0 (0.0)	0.2 (0.7)	0.0 (0.0)	9.1 (7.1)	0.0 (0.0)	1.2 (2.6)	0.0 (0.0)	0.0 (0.0)
St	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.5 (6.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Br	0.0 (0.0)	0.4 (1.9)	0.2 (1.1)	0.0 (0.0)	0.3 (1.4)	0.0 (0.0)	1.0 (2.4)	0.0 (0.0)	0.6 (2.8)	0.0 (0.0)	0.0 (0.0)
Yu	1.3 (3.4)	0.0 (0.0)	1.0 (2.2)	0.0 (0.0)	3.3 (5.6)	0.0 (0.0)	4.9 (7.6)	0.0 (0.0)	2.2 (5.0)	0.0 (0.0)	0.0 (0.0)
Ka	0.5 (2.5)	0.0 (0.0)	0.2 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.6 (7.8)	0.0 (0.0)	0.3 (1.2)	0.0 (0.0)	0.0 (0.0)

Note: In each simulation, a 50-fish random sample was generated from the baseline stock and the stock composition was estimated with a 22-stock resampled baseline. Twenty-five simulated pure (100%) test samples were derived for each stock, and the mean and standard deviation (in parentheses) are reported. Stock abbreviations are as in Table 1.

and Wright 1993) indicates that the DNA sequences interspersed among the VNTR regions may represent a flanking sequence from the original Ssa1 locus that in coho salmon has been replicated along with the VNTR sequence in some tandem fashion. Although this is an unusual construction for a minisatellite locus, it is not novel, as minisatellite loci with internal restriction sites have also been detected in dogs (Jeffreys and Morton 1987) and swans (Meng et al. 1990) using human minisatellite probes. A study to further elucidate the nature of the Ssa1 locus in various salmonid species is currently underway in our laboratory.

In Atlantic salmon, from which the Ssa1 probe was isolated, Ssa1 alleles are apparently single DNA fragments 3.5–6.0 kbp in size (Bentzen and Wright 1993), indicating that in this species tandem duplication of the VNTR region plus flanking DNA has not occurred. Nine Ssa1 alleles were detected in 77 Atlantic salmon from three different populations, among which mean heterozygosity ranged from 0.36 to 0.77 (Bentzen and Wright 1993). However, the multiplicated nature of the Ssa1 locus in coho may provide more opportunity for variation than in the single repeat locus of Atlantic salmon. We detected seven alleles in six coho salmon derived from the Kitimat River population, and five of the six fish were heterozygous at Ssa1. Moreover, the strong differentiation of stocks provided by the Ssa1 data set of this study indicates that Ssa1 allelic frequencies vary greatly among coho salmon populations. To what extent the high level of Ssa1 polymorphism in coho salmon is due to variability in the number of repeat units between conserved pairs of restriction sites as opposed to variability in the number of tandem duplications of the original Ssa1 locus is not clear. If each VNTR region within the coho salmon Ssa1 locus is subject to the high rate of mutation characteristic of minisatellite loci, the level of polymorphism detected by Ssa1 in this species may more closely resemble that detected by multilocus minisatellite probes in other organisms than the moderate level described for the Atlantic salmon Ssa1 locus (Bentzen and Wright 1993).

Although the pedigree analysis showed that Ssa1 recognizes a single locus in coho, over the entire data set fragments belonging to a given allele could not be identified without a segregation study for every allele. Considering that seven alleles were identified among the six Kitimat fish used in the pedigree analysis, the number of alleles sampled over all 975 fish from 22 populations is quite high. Therefore, these data could not be put into the traditional format of allelic frequencies used in electrophoretic or single locus DNA data. Instead, data were analyzed in the same manner as multilocus DNA fingerprint data, specifically as the number of restriction fragments in 205 bins for each fish over all five restriction enzymes. The analysis of bin data can be treated similarly to allelic frequency data. However, because in this particular data set, bins are not independent of one another, and since an allele is made up of multiple bins (fragments), in the analysis, they were converted to independent variables using principal components analysis. We were then able to use the traditional maximum likelihood method of analysis used in electrophoretic or single locus DNA data to estimate stock compositions.

Of the three coho salmon families used in the pedigree analysis, the Ssa1 probe detected the same locus in DNA restricted with all four restriction enzymes. Thus, theoretically, no additional genetic information about this locus was gained

Te	Ca	Co	Sa	Ea	Che	Chi	St	Br	Yu	Ka
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (1.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.2 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.6 (2.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.9 (3.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (2.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.3 (2.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.4 (2.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.8 (2.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.1 (3.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (1.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
80.4 (7.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (2.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	90.5 (6.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	92.4 (7.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	86.3 (11.3)	0.0 (0.0)	0.0 (0.0)	0.4 (1.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	89.0 (6.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	94.9 (5.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	86.5 (7.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	97.5 (6.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	97.5 (4.2)	0.0 (0.0)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.9 (2.2)	0.0 (0.0)	0.0 (0.0)	86.3 (10.1)	0.0 (0.0)
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (1.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	92.0 (8.4)

Table 6. Estimated percentage compositions of simulated mixtures of stocks containing 50 individuals from the Strait of Georgia tested against fixed and resampled 22-stock baselines.

Stock	Correct	Fixed	Resampled	Correct	Fixed	Resampled
Big Qualicum	50	50.0 (0.0)	46.6 (4.2)	10	10.0 (0.0)	10.3 (3.9)
Quinsam	20	19.5 (1.0)	22.2 (4.8)	50	48.7 (1.7)	50.3 (7.9)
Nanaimo	10	10.0 (0.0)	9.4 (3.1)	20	20.0 (0.0)	17.2 (5.4)
Tenderfoot	10	10.5 (1.0)	8.2 (3.3)	10	11.3 (1.7)	9.6 (3.0)
Puntledge	10	10.0 (0.0)	8.5 (2.5)	10	10.0 (0.0)	7.7 (2.6)

Note: Means and standard deviation (of 25 simulated mixtures) are shown.

Table 7. Estimated percentage compositions of simulated mixtures of stocks containing 50 individuals from various stocks on the British Columbia coast tested against fixed and resampled 22-stock baselines.

Stock	Correct	Fixed	Resampled
Kitimat 50	50	50.0 (0.0)	43.1 (5.6)
Atnarko 20	20	20.0 (0.0)	17.5 (4.0)
Quinsam	10	9.8 (0.7)	17.8 (6.3)
Chilliwack	10	10.0 (0.0)	9.9 (4.0)
Robertson	10	10.0 (0.0)	9.7 (4.7)

Note: Means and standard deviations of 25 simulated mixtures are shown.

by the use of more than one restriction enzyme. In fact, the data sets of fragment number and molecular size generated from the different restriction enzymes provided different patterns of population resolution. This was evident in the comparisons among stocks, for which some enzymes provided better discrimination than others, and in the comparisons of multiple samples of the same stock, for which some enzymes revealed differences not detected by others. There are a number of factors that probably contributed to the different patterns provided by DNA restricted with different enzymes. The data were not analyzed as single locus allelic frequencies, but as DNA fragment size and frequency. Our pedigree analysis showed that different Ssa1 alleles often shared restriction fragments, but the same alleles did not always share fragments over all restriction enzymes. Thus, two populations of coho salmon characterized by two different Ssa1 alleles would be better discriminated by data generated from a restriction enzyme producing banding patterns in which the two alleles did not share DNA fragments than by data generated from a restriction enzyme producing banding patterns in which those particular alleles shared one or more DNA fragments. We also showed that different Ssa1 alleles very similar in size are not necessarily detectable in the banding patterns provided by all restriction enzymes. A small (<500 bp) difference in fragment

Table 8. Estimated percentage compositions of three simulated mixtures of stocks containing 200 fish from various regions on the southern British Columbia coast tested against fixed and resampled 14-stock baselines.

Stock	Correct	Fixed	Resampled	Correct	Fixed	Resampled	Correct	Fixed	Boot
Robertson	25	25.0 (0.0)	23.1 (1.8)	10	10.0 (0.0)	10.1 (1.5)	0	—	0.6 (0.9)
Nitinat	20	20.0 (0.0)	18.0 (2.0)	0	—	—	0	—	—
Quinsam	20	19.5 (0.4)	26.8 (3.8)	30	29.3 (0.5)	34.4 (3.0)	20	19.7 (0.5)	24.7 (3.2)
Puntledge	0	—	—	20	20.0 (0.0)	18.0 (2.0)	0	—	—
Big Qualicum	5	5.0 (0.0)	5.4 (1.4)	10	10.0 (0.0)	10.4 (1.9)	10	10.0 (0.0)	9.7 (1.9)
Nanaimo	0	—	—	0	—	—	0	—	—
Shawnigan	0	—	—	0	—	—	0	—	—
Tenderfoot	0	0.5 (0.4)	—	0	0.7 (0.5)	—	0	0.3 (0.5)	—
Capilano	10	10.0 (0.0)	8.7 (1.9)	10	10.0 (0.0)	9.1 (1.7)	0	—	—
Coldwater	0	—	—	10	10.0 (0.0)	8.8 (1.5)	10	10.0 (0.0)	9.8 (1.5)
Salmon	0	—	—	0	—	—	10	10.0 (0.0)	9.7 (1.3)
Eagle	0	—	—	0	—	—	10	10.0 (0.0)	8.9 (2.0)
Chehalis	10	10.0 (0.0)	9.2 (1.1)	0	—	—	20	20.0 (0.0)	17.4 (2.8
Chilliwack	10	10.0 (0.0)	8.9 (1.5)	10	10.0 (0.0)	9.1 (1.6)	20	20.0 (0.0)	19.1 (2.3)
WCVI	45	45.0 (0.0)	41.0 (3.0)	10	10.0 (0.0)	10.1 (1.7)	0	—	0.6 (0.0)
SGEORG	35	35.0 (0.0)	41.0 (2.3)	70	70.0 (0.0)	72.0 (2.70	30	30.0 (0.0)	34.5 (3.6)
FRASER	20	20.0 (0.0)	18.0 (1.7)	20	20.0 (0.0)	17.9 (2.3)	70	70.0 (0.0)	64.9 (3.4)

Note: Means and standard deviations of 25 simulated mixtures are shown, with regional summaries at the bottom of the table. WCVI, west coast Vancouver Island; SGEORG, Strait of Georgia.

Table 9. Estimated percentage compositions of simulated pure stocks containing 50 individuals from the Fraser River, tested against a resampled baseline of five Fraser River stocks.

	Salmon	Eagle	Coldwater	Chehalis	Chilliwack
Salmon	99.7 (1.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (1.4)
Eagle	0.0 (0.0)	98.5 (2.6)	0.0 (0.0)	0.0 (0.0)	1.5 (2.6)
Coldwater	0.0 (0.0)	0.0 (0.0)	99.6 (1.7)	0.0 (0.0)	0.4 (1.7)
Chehalis	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	99.3 (2.0)	0.7 (2.0)
Chilliwack	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (0.0)

Note: Means and standard deviations of 25 simulations per stock are shown.

size can go undetected if it occurs in a DNA fragment less than 2.0 kbp in size (not scored in this study), or in a DNA fragment greater than 12.0 kbp in size (band resolution too poor to detect difference). In this study, one Ssa1 allele was differentiated from a very similar allele only in the *Hin*fI-restricted DNA of a coho salmon family; the allele went undetected in the banding patterns of DNA produced by restriction with three other enzymes.

Regional definition of North Pacific coho salmon

Although genetic distances between stocks of coho salmon were high, the geographic distances among populations within British Columbia were not necessarily positively associated with the D^2 values. As a result, regional definition between populations in British Columbia was weak. However, most of the stocks from the Fraser River were distinct among other British Columbia stocks, and further grouped into upper and lower Fraser River categories.

Regional definition of coho stocks did occur on a larger geographic scale, with stocks north of British Columbia forming a distinct group from British Columbia stocks. Therefore, although stocks within the lower coastal mainland, Vancouver Island, and northern British Columbia did not form regional groups on the basis of Ssa1 polymorphism on a small scale, some large-scale definition occurred. Further, as demonstrated by the large D^2 values between stocks, many coho stocks within British Columbia are distinct and separable from one another. This distinctiveness between stocks resulted in the ability to identify fish to specific stocks and to estimate stock compositions within British Columbia with high levels of accuracy. Furthermore, the discrete nature of the stocks suggests that intrastock distribution of new alleles occurs rapidly, and faster than straying can exchange them between stocks.

Discrimination of coho stocks and stock compositions

Using linear discriminant analysis, we were able to correctly identify 45% of jackknifed test fish to their stocks of origin with a baseline data set of 22 stocks. Furthermore, fish were identified to specific stocks within British Columbia with as high as 82% accuracy. Finally, although low levels of interannual variation were detected in some British Columbia stocks, 62% of fish from the stock with the highest levels of interannual variation, Robertson Creek, were correctly identified to stock when annual data were combined.

Ssa1 minisatellite variation enabled highly accurate estimations of stock compositions of coho salmon from multistock mixtures. Bias of the individual stock estimates will be greatest when estimates comprise a very small or very large portion of the mixture, given the constraints on the maximum likelihood estimator. However, even when single-stock samples were simulated and resolved with a resampled baseline, estimates of stock composition were up to 98% with a 22-stock baseline and 100% for a 5-stock Fraser River baseline. Stock proportions estimated using mixed-stock test samples were on average within 1.4% (14-stock resampled baseline) to 2.4% (22-stock resampled baseline) of the actual proportions.

We have shown that variation exists between populations (or stocks) at the Ssa1 locus, and that this variation can be used

to quite accurately identify fish to some stocks and to estimate stock compositions in mixed fishery samples. However, greater numbers of fish per stock should be sampled, as well as a greater number of stocks, before the usefulness of the Ssa1 locus to fisheries managers is known. Highly accurate stock identification in the future will likely result from the use of multiple polymorphic loci, most likely containing mini- and micro-satellites and possibly some major histocompatability genes.

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Appendix

Mean bin counts at the Ssa1 locus for 22 stocks of coho salmon.

Stock	*N*	1	2	3	4	5	6	7	8	9	10	11
Atnarko	79	0.000	0.177	0.658	0.038	0.000	0.000	0.013	0.013	0.000	0.203	0.316
Kitimat	100	0.030	0.200	0.630	0.010	0.010	0.010	0.080	0.040	0.000	0.260	0.320
Pallant	93	0.011	0.226	0.667	0.151	0.000	0.000	0.000	0.000	0.000	0.054	0.269
Toboggan	28	0.000	0.393	0.750	0.000	0.000	0.000	0.036	0.107	0.036	0.000	0.000
Stikine	9	0.000	0.111	0.889	0.333	0.000	0.000	0.111	0.000	0.000	0.333	0.222
Robertson	75	0.000	0.253	0.720	0.240	0.000	0.000	0.027	0.013	0.000	0.107	0.040
Nitinat	44	0.136	0.136	0.591	0.068	0.000	0.023	0.159	0.000	0.000	0.045	0.205
Quinsam	104	0.029	0.154	0.558	0.048	0.010	0.019	0.115	0.048	0.067	0.096	0.173
Puntledge	39	0.000	0.154	0.615	0.179	0.026	0.000	0.000	0.000	0.000	0.051	0.154
Big Qualicum	75	0.013	0.200	0.347	0.173	0.013	0.000	0.013	0.000	0.013	0.000	0.133
Nanaimo	28	0.000	0.071	0.286	0.000	0.000	0.036	0.036	0.036	0.000	0.000	0.000
Shawnigan	47	0.000	0.149	0.957	0.021	0.000	0.000	0.000	0.128	0.000	0.170	0.000
Tenderfoot	52	0.000	0.077	0.538	0.058	0.000	0.000	0.077	0.019	0.000	0.154	0.212
Capilano	42	0.000	0.190	0.714	0.048	0.000	0.000	0.214	0.119	0.000	0.071	0.262
Coldwater	31	0.097	0.000	0.323	0.000	0.097	0.194	0.355	0.032	0.161	0.032	0.226
Salmon	16	0.000	0.000	0.375	0.125	0.000	0.000	0.563	0.000	0.000	0.063	0.000
Eagle	46	0.130	0.130	0.696	0.043	0.087	0.000	0.674	0.652	0.065	0.196	0.152
Chehalis	32	0.000	0.063	0.531	0.000	0.031	0.031	0.375	0.031	0.031	0.000	0.156
Chilliwack	82	0.012	0.171	0.439	0.049	0.024	0.037	0.439	0.049	0.037	0.098	0.134
Bristol Bay	25	0.040	0.040	1.000	0.040	0.040	0.000	0.000	0.000	0.040	0.160	0.000
Yukon	18	0.000	0.167	1.056	0.056	0.000	0.000	0.056	0.000	0.000	0.000	0.000
Kamchatka	30	0.033	0.133	0.667	0.100	0.033	0.033	0.000	0.033	0.200	0.267	0.033

Stock	*N*	22	23	24	25	26	27	28	29	30	31
Atnarko	79	0.013	0.000	0.114	0.165	0.114	0.114	0.380	0.114	0.025	0.013
Kitimat	100	0.030	0.030	0.140	0.200	0.060	0.070	0.520	0.070	0.030	0.020
Pallant	93	0.054	0.226	0.043	0.065	0.022	0.000	0.323	0.247	0.118	0.086
Toboggan	28	0.036	0.071	0.143	0.250	0.000	0.107	0.036	0.143	0.000	0.000
Stikine	9	0.000	0.000	0.222	0.111	0.000	0.000	0.222	0.000	0.222	0.111
Robertson	75	0.013	0.053	0.093	0.027	0.000	0.000	0.200	0.093	0.067	0.147
Nitinat	44	0.000	0.000	0.455	0.091	0.000	0.045	0.114	0.068	0.000	0.114
Quinsam	104	0.067	0.058	0.106	0.115	0.135	0.135	0.288	0.077	0.087	0.144
Puntledge	39	0.154	0.026	0.154	0.103	0.000	0.000	0.154	0.000	0.077	0.000
Big Qualicum	75	0.040	0.013	0.120	0.200	0.027	0.000	0.200	0.107	0.027	0.147
Nanaimo	28	0.286	0.036	0.179	0.071	0.036	0.000	0.071	0.000	0.071	0.107
Shawnigan	47	0.106	0.021	0.064	0.128	0.106	0.000	0.106	0.000	0.000	0.021
Tenderfoot	52	0.135	0.019	0.154	0.096	0.019	0.077	0.327	0.058	0.019	0.096
Capilano	42	0.095	0.119	0.095	0.310	0.119	0.214	0.143	0.095	0.000	0.000
Coldwater	31	0.097	0.129	0.161	0.290	0.000	0.161	0.323	0.032	0.000	0.290
Salmon	16	0.188	0.000	0.563	0.000	0.125	0.125	0.000	0.000	0.000	0.188
Eagle	46	0.109	0.043	0.370	0.391	0.022	0.065	0.000	0.043	0.000	0.152
Chehalis	32	0.188	0.094	0.219	0.344	0.063	0.094	0.156	0.156	0.000	0.250
Chilliwack	82	0.098	0.012	0.134	0.341	0.085	0.122	0.110	0.110	0.049	0.207
Bristol Bay	25	0.040	0.080	0.040	0.040	0.040	0.000	0.040	0.160	0.040	0.120
Yukon	18	0.167	0.056	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.056
Kamchatka	30	0.000	0.067	0.167	0.333	0.000	0.000	0.033	0.067	0.133	0.067

Note: Genomic DNA was restricted with *Mbol* and hybridized with the Ssa1 probe. Molecular weight intervals for definition of bins were as follows: 1.94–2.01 kb (1), 2.02–2.26 (2), 2.27–2.42 (3), 2.43–2.59 (4), 2.60–2.76 (5), 2.77–2.94 (6), 2.95–3.12 (7), 3.13–3.31 (8), 3.32–3.50 (9), 3.51–3.70 (10), 3.71–3.91(11), 3.92–4.12 (12), 4.13–4.34 (13), 4.35–4.56 (14), 4.57–4.80 (15), 4.81–5.04 (16), 5.05–5.29 (17), 5.30–5.55 (18), 5.56–5.83 (19), 5.84–6.11 (20), 6.12–6.41 (21), 6.42–6.72 (22), 6.73–7.05 (23), 7.06–7.39 (24), 7.40–7.75 (25), 7.76–8.13 (26), 8.14–8.54 (27), 8.55–8.97 (28), 8.98–9.44 (29), 9.45–9.93 (30), 9.94–10.47 (31), 10.48–11.06 (32), 11.07–11.70 (33), 11.71–12.42 (34), 12.43–13.22 (35), 13.23–14.12 (36), 14.13–15.18 (37), 15.19–16.43 (38), 16.44–17.99 (39), 18.00–20.02 (40), and 20.03–23.00 (41). *N* is sample size.

12	13	14	15	16	17	18	19	20	21
0.367	0.013	0.000	0.013	0.025	0.747	0.025	0.532	0.595	0.481
0.310	0.000	0.010	0.010	0.070	0.610	0.020	0.540	0.630	0.410
0.312	0.011	0.011	0.011	0.000	0.656	0.043	0.452	0.785	0.333
0.143	0.036	0.000	0.000	0.143	0.536	0.107	0.464	0.679	0.286
0.000	0.000	0.000	0.000	0.000	0.556	0.000	0.667	0.556	0.111
0.187	0.000	0.000	0.000	0.027	0.573	0.320	0.573	0.507	0.493
0.250	0.023	0.000	0.000	0.000	0.682	0.045	0.318	0.477	0.250
0.375	0.106	0.000	0.019	0.144	0.673	0.010	0.394	0.538	0.490
0.103	0.000	0.000	0.000	0.051	0.641	0.051	0.487	0.513	0.590
0.320	0.080	0.000	0.000	0.200	0.613	0.173	0.280	0.653	0.267
0.286	0.036	0.000	0.000	0.107	0.857	0.071	0.571	0.393	0.393
0.106	0.000	0.000	0.021	0.000	0.319	0.021	0.489	0.532	0.468
0.365	0.038	0.019	0.000	0.019	0.846	0.000	0.558	0.558	0.462
0.167	0.000	0.024	0.024	0.048	0.833	0.048	0.476	0.690	0.476
0.226	0.000	0.032	0.000	0.032	0.742	0.000	0.452	0.516	0.032
0.188	0.000	0.000	0.313	0.000	0.500	0.000	0.563	0.125	0.063
0.196	0.174	0.022	0.087	0.022	0.370	0.000	0.326	0.239	0.065
0.156	0.000	0.000	0.063	0.188	0.563	0.031	0.563	0.625	0.281
0.280	0.000	0.000	0.000	0.012	0.744	0.000	0.354	0.768	0.280
0.040	0.080	0.000	0.040	0.000	0.880	0.120	0.920	0.160	0.520
0.056	0.056	0.056	0.000	0.000	0.722	0.111	0.944	0.111	0.722
0.000	0.200	0.000	0.033	0.033	0.400	0.000	0.233	0.433	0.367

32	33	34	35	36	37	38	39	40	41
0.076	0.203	0.266	0.253	0.278	0.228	0.190	0.089	0.051	0.063
0.100	0.230	0.300	0.320	0.190	0.090	0.070	0.080	0.100	0.050
0.043	0.108	0.226	0.398	0.269	0.194	0.032	0.043	0.043	0.086
0.143	0.214	0.143	0.179	0.286	0.179	0.179	0.000	0.143	0.107
0.444	0.111	0.111	0.111	0.000	0.000	0.333	0.000	0.111	0.000
0.227	0.213	0.187	0.187	0.053	0.187	0.093	0.253	0.307	0.200
0.114	0.136	0.182	0.341	0.205	0.182	0.045	0.227	0.159	0.045
0.173	0.144	0.269	0.375	0.144	0.144	0.067	0.058	0.096	0.096
0.231	0.026	0.282	0.282	0.205	0.051	0.256	0.026	0.128	0.103
0.067	0.227	0.333	0.280	0.200	0.147	0.020	0.027	0.107	0.120
0.143	0.179	0.170	0.250	0.286	0.143	0.321	0.179	0.071	0.036
0.298	0.277	0.234	0.383	0.149	0.021	0.191	0.000	0.085	0.170
0.135	0.154	0.096	0.327	0.231	0.154	0.231	0.115	0.096	0.077
0.095	0.024	0.119	0.310	0.262	0.214	0.333	0.071	0.095	0.048
0.194	0.097	0.065	0.548	0.129	0.032	0.129	0.000	0.226	0.000
0.375	0.188	0.000	0.563	0.063	0.063	0.125	0.000	0.375	0.063
0.087	0.065	0.391	0.239	0.152	0.174	0.152	0.022	0.109	0.130
0.094	0.063	0.156	0.375	0.156	0.219	0.156	0.094	0.094	0.156
0.220	0.159	0.110	0.341	0.171	0.171	0.110	0.049	0.110	0.134
0.000	0.080	0.080	0.160	0.040	0.400	0.080	0.120	0.120	0.000
0.000	0.111	0.167	0.222	0.000	0.389	0.056	0.333	0.056	0.000
0.100	0.167	0.100	0.167	0.100	0.267	0.200	0.000	0.133	0.000

Regression of abundance estimates from mark–recapture surveys against environmental covariates

John R. Skalski

Abstract: Two alternative approaches are presented for regressing abundance estimates from mark–recapture surveys against environmental covariates. The first is based on weighted least squares under the assumption of normality of square-root transformed estimates of abundance. A second approach based on maximum likelihood estimation and analysis of deviance is presented that inherently weights the precision of the survey results and is relatively distribution free. The analytic approaches are illustrated using a study whose objective is to relate 8 years of abundance estimates of chinook salmon smolt production versus spring river flow conditions. Analysis of deviance and maximum likelihood estimation procedures are recommended for the analysis of replicated mark–recapture studies because they intrinsically provide proper weighting of the capture data and an opportunity to construct realistic response models independent of distributional assumptions of the data.

Résumé : Nous présentons deux nouvelles méthodes pour calculer la régression des estimations de l'abondance à partir des études de marquage–recapture en fonction des covariables environnementales. La première se fonde sur les moindres carrés pondérés dans l'hypothèse de normalité des estimations de l'abondance après transformation quadratique. La deuxième méthode présentée, qui se fonde sur l'estimation du maximum de vraisemblance et sur l'analyse de déviance, pondère automatiquement la précision des résultats et est relativement indépendante de la distribution. Ces méthodes d'analyse sont illustrées dans une étude dont l'objectif est de mettre en rapport 8 ans d'estimation de l'abondance de la production de smolts de quinnat avec les conditions de débit d'une rivière au printemps. L'analyse de déviance et l'estimation du maximum de vraisemblance sont recommandées pour l'analyse des études de marquage–recapture répétées parce qu'elles assurent de façon intrinsèque une pondération correcte des données sur les captures et la possibilité de construire des modèles réalistes de réponse qui soient indépendants des hypothèses de distribution des données.
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Introduction

Mark–recapture methods serve a central role in quantifying the abundance, movement, and survival of mobile species (Seber 1982). Despite the costs and logistical difficulties of undertaking mark–recapture studies, the use of fish tagging continues to increase. One area of phenomenal growth has been the use of coded wire tags (CWT) to mark salmonids on the Pacific coast of United States and Canada. An estimated 43.6 million salmon smolt were marked with CWT in 1990 alone (Johnson and Longwill 1991). Among the objectives of these tagging studies are estimation of smolt production and survival rates, catch apportionment, and comparisons of fishing stocks and hatchery rearing practices. The widespread use of the tagging method has encouraged comparative investigations in the fishery.

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Analysis of comparative studies of animal abundance has recently received direct attention (Skalski and Robson 1992). However, regression techniques for specific use in conjunction with mark–recapture estimates of animal abundance have not been developed. The distributional nature of abundance estimates typically does not satisfy the assumptions of normality and constant variance of ordinary least squares. For this reason, alternative approaches for analyzing animal abundance data are needed that conform to the characteristics of abundance estimates. A comparative study of the relationship between annual production of wild chinook salmon smolt and ambient river conditions will be illustrated. The purpose of this fishery investigation was to help establish flow management guidelines to protect salmon production.

The objective of this paper is to compare the prevailing approach to analysis using weighted least squares (WLS) with the techniques of maximum likelihood estimation (MLE) and analysis of deviance (ANODEV). Using the CWT data from the smolt production study, two alternative regression techniques are presented. The WLS technique is based on approximate normality of square-root transformed abundance estimates, while the MLE approach is based on the asymptotic chi-square distribution of likelihood ratio tests and F

Table 1. Numbers of smolt marked (n_1), adult captures (n_2), number of mark–recaptures (m), abundance estimates ($\hat{N}$), standard errors of the abundance estimates (SE($\hat{N}$)), coefficient of variation (CV), and weights used in weighted least squares analyses.

Brood year	n_1	n_2	m	$\hat{N}$	SE ($\hat{N}$)	CV (%)	Weight (w)
1977	35 866	3069	42	2 560 736	383 103	15.0	0.000 016 3
1978	78 083	1615	45	2 743 124	394 274	14.4	0.000 016 5
1979	103 796	2984	130	2 365 144	201 165	8.5	0.000 057 1
1982	96 443	2869	96	2 853 548	283 197	9.9	0.000 034 5
1983	101 270	3950	86	4 599 099	484 629	10.5	0.000 018 9
1984	69 533	2981	55	3 702 685	485 610	13.1	0.000 014 9
1985	84 774	1630	46	2 941 872	418 344	14.2	0.000 015 8
1986	83 340	656	25	2 105 962	397 130	18.9	0.000 011 9

distribution for ratios of independent chi-square statistics. Extension of these methods to other statistical problems will be discussed.

Example: juvenile chinook production

The Washington Department of Fisheries conducted tagging studies of juvenile fall chinook (*Oncohynchus tshawytscha*) in the North Fork Lewis River for the brood years 1977–1979 and 1982–1986 (Norman et al. 1987). Juvenile salmon were tagged in spring and recaptured when the individuals returned as adults at 2–6 years of age. The objective of the study was to relate juvenile production to river flow conditions during spring runoff (May–July) for each brood year. The capture data presented (Table 1) ignore measurement errors associated with tag loss and possible misclassification of adult spawners to brood year (Skalski 1989) to focus attention on the regression techniques. Consequently, the regression analyses are for illustrative purposes only and do not necessarily represent a thorough evaluation of the data or the conclusions of interested parties.

The single mark–recapture data used in estimating the smolt production for a particular brood year can be modeled as a multinomial likelihood (Skalski and Robson 1992)

$$[1]\quad L(N, p_1, p_2 | n_1, n_2, m) = \binom{n_1 - m, \; N}{n_2 - m, \; m} (p_1 q_2)^{n_1 - m} (q_1 p_2)^{n_2 - m} (p_1 p_2)^{m} (q_1 q_2)^{N - r}$$

where N is juvenile abundance, $p_1 = 1 - q_1$ is the probability that a smolt is captured in the initial marking period, $p_2 = 1 - q_2$ is the probability that an adult salmon is captured in the recovery period, n_1 is the number of smolt of a brood year tagged, n_2 is the number of adults of a brood year examined for tags, m is the number of adults of a brood year recovered with CWT, and $r = n_1 + n_2 - m$ is the number of distinct fish captured for a brood year.

The maximum likelihood estimator of abundance ($\hat{N}$) is the traditional estimate for Petersen single mark-recapture data. However, juvenile abundance will be estimated instead by the Chapman (1951) modification that is almost unbiased (i.e., unbiased when $n_1 + n_2 \geq N$) where

$$[2]\quad \hat{N} = \frac{(n_1 + 1)(n_2 + 1)}{m + 1} - 1.$$

The variance of the abundance estimate is approximately

$$[3]\quad \mathrm{Var}(\hat{N}|N) = \frac{N q_1 q_2}{p_1 p_2}$$

with estimated variance (Seber 1982, p. 60)

$$[4]\quad \hat{\mathrm{Var}}(\hat{N}|N) = \frac{(n_1 + 1)(n_2 + 1)(n_1 - m)(n_2 - m)}{(m + 1)^2 (m + 2)}.$$

Equation 4 is used to estimate the sampling error for the data collected, while eq. 3 will be used to suggest what type of weighting may be appropriate in subsequent regression analyses. Estimates of juvenile chinook production for brood years 1977–1979 and 1982–1986 and associated standard errors (SE) are given in Table 1.

Among the independent variables of interest are characterizations of river flow conditions during the late spring – summer season (1 May – 31 July). A problem confronting the regression analysis is that each spring season provides 85 days of river flow measurements and numerous ways of characterizing the flow patterns (e.g., minimum, maximum, mean, and median daily flows by season or month) when regressing against the eight juvenile production estimates. With sufficient ingenuity and effort, finding a statistically significant production–flow relationship is almost assured. Fisher (1924) suggested a solution to the problem of subjectively choosing among numerous formulations for the independent variables when faced with analyzing 60 years of wheat production at Rothamsted against daily rainfall measurements for the same period. The pattern of daily measurements is first smoothed using any of a number of smoothing techniques (e.g., period averages, running averages, Tukey smoothing algorithms (Tukey 1977, pp. 205–236)) followed by modeling the temporal pattern using polynomial regression as a function of time. The results of the polynomial regression provide an objective criterion for selection of summary statistics for the daily flow measurements. For this example, average 5-day flows on the North Fork Lewis River were computed for the period (1 May –29 July) and regressed against time. The highest order polynomials that significantly characterized annual flow patterns were either linear and quadratic functions in time where

$$[5]\quad f_i = \beta_0 + \beta_1 t_i$$

or

$$[6]\quad f_i = \beta_0 + \beta_1 t_i + \beta_2 t_i^2$$

and where f_i is the average flow for the ith 5-day period ($i = 0$,

Table 2. Regression coefficients for linear and quadratic models of river flow as a function of time period.

		Linear (equation 2)			Quadratic (equation 3)			
Brood year	Spring flow	β_{L_0}	β_{L_1}	r^2	β_{Q_0}	β_{Q_1}	β_{Q_2}	r^2
1977	1978	4400	−187.9	0.658	4524	−234.2	2.72	0.661
1978	1979	3799	−156.1	0.440	4713	−498.8	20.16	0.596
1979	1980	5455	−293.3	0.589	5262	−219.7	−4.28	0.592
1982	1983	2677	26.1	0.050	3109	−136.2	9.55	0.193
1983	1984	6393	−271.5	0.750	5720	−19.11	−14.85	0.797
1984	1985	7100	−351.4	0.593	6200	−13.72	−19.87	0.634
1985	1986	4141	−213.7	0.803	4859	−482.8	15.83	0.897
1986	1987	2276	−63.1	0.830	2104	1.6	−3.80	0.894

1, . . ., 17) and t_i is the sequential indicator of the ith 5-day time period ($t_i = 0, 1, 2, \ldots 17$).

Patterns of daily flows (Fig. 1) and associated regression coefficients (Table 2) were characterized by year. Values of r^2 for a linear model (eq. 5) in time ranged from 0.050 to 0.830 with an average value of 0.589. For the quadratic model (eq. 6), r^2 ranged from 0.193 to 0.897 with an average value of 0.658. Addition of a quadratic term to the model increased the overall r^2 for the fit from 0.003 to 0.156 (Table 2). Only during the spring flow years 1979, 1986, and 1987 was a quadratic trend evident. The regression coefficients calculated for each year (Table 2) serve as the independent variables used in the subsequent regression analysis of smolt production. For convenience of notation, regression coefficients associated with the linear flow model are denoted β_{L_0} and β_{L_1} whereas coefficients for the quadratic flow model are denoted β_{Q_0}, β_{Q_1}, and β_{Q_2}.

In the case of the linear flow relationship (eq. 5), β_{L_0} can be readily interpreted as the level of river flow at the beginning of the spring season and β_{L_1} as the rate of change in that flow over the course of the season. The regression coefficients for the quadratic relationship (i.e., β_{Q_0}, β_{Q_1} and β_{Q_2}) have a somewhat analogous interpretation. Note (Table 2) that the β_{L_0} and β_{L_1} values for the linear flow model are negatively correlated ($r = -0.926$). This negative correlation is induced in part by the relatively stable summer flow levels following the spring freshet.

The calculation of the regression coefficients for the river flow pattern is a special case of regression estimation in finite sampling theory (Cochran 1977, p. 191). In the case of linear regression, β_{L_1} is defined as

$$\beta_{L_1} = \frac{\sum_{i=1}^{K} (y_i - \bar{Y})(x_i - \bar{X})}{\sum_{i=1}^{K} (x_i - \bar{X})^2}$$

where x_i and y_i are the independent and dependent variables, respectively, and K is the number of sampling units in the entire population. In the case of river flow patterns where sample size $k = K$ (17 for moving averages or 85 daily observations), the variances of the regression coefficient are zero, which avoids the situation of an errors in variables regression (Snedecor and Cochran 1989, pp. 173–174). It should be noted that $\mathrm{Var}(\beta_{L_1}) = 0$ in finite regression does not necessarily imply that the data perfectly match the model predictions; rather, the population of observations has been totally enumerated and characterized. Calculating the regression coefficients for river flow is wholly analogous to calculating the average grade of a stretch of road. If the entire road is measured, the average grade is calculated without measurement error despite the fact the road may have some dips and rises.

Methods

WLS and MLE methods followed by ANODEV were used to investigate the smolt production – river flow relationships.

Weighted least squares

The eight abundance estimates (Table 1) serve as the dependent variables in the multiple regression analysis against the polynomial regression coefficients extracted from the seasonal river flow patterns. The MLEs of abundance are asymptotically normally distributed. However, the sampling error (eq. 3) of the abundance estimates is a function of abundance itself (N) and the per-period capture rates (p_1 and p_2). Furthermore, the sampling distribution for $\hat{N}$ is skewed to the right and is truncated to the left at the value $r = n_1 + n_2 - m$. Hence, the abundance estimates are not normally distributed, nor do they have equal variance.

A square-root transformation of the $\hat{N}$ results in a sampling distribution that is less skewed with approximate variance

$$[7] \quad \mathrm{Var}(\sqrt{\hat{N}} \mid N) \approx \frac{\mathrm{Var}(\hat{N} \mid N)}{4N} = \frac{q_1 q_2}{4 p_1 p_2}$$

independent of N. Despite the transformation, the variances may be still unequal, requiring WLS with weights inversely proportioned to eq. 7. Substituting in the MLEs for p_1 and p_2, (i.e., $\hat{p}_1 = \frac{m}{n_2}$ and $\hat{p}_2 = \frac{m}{n_1}$) the weights are expressible as

$$[8] \quad w_i = \frac{m_i^2}{(n_{2i} - m_i)(n_{1i} - m_i)}$$

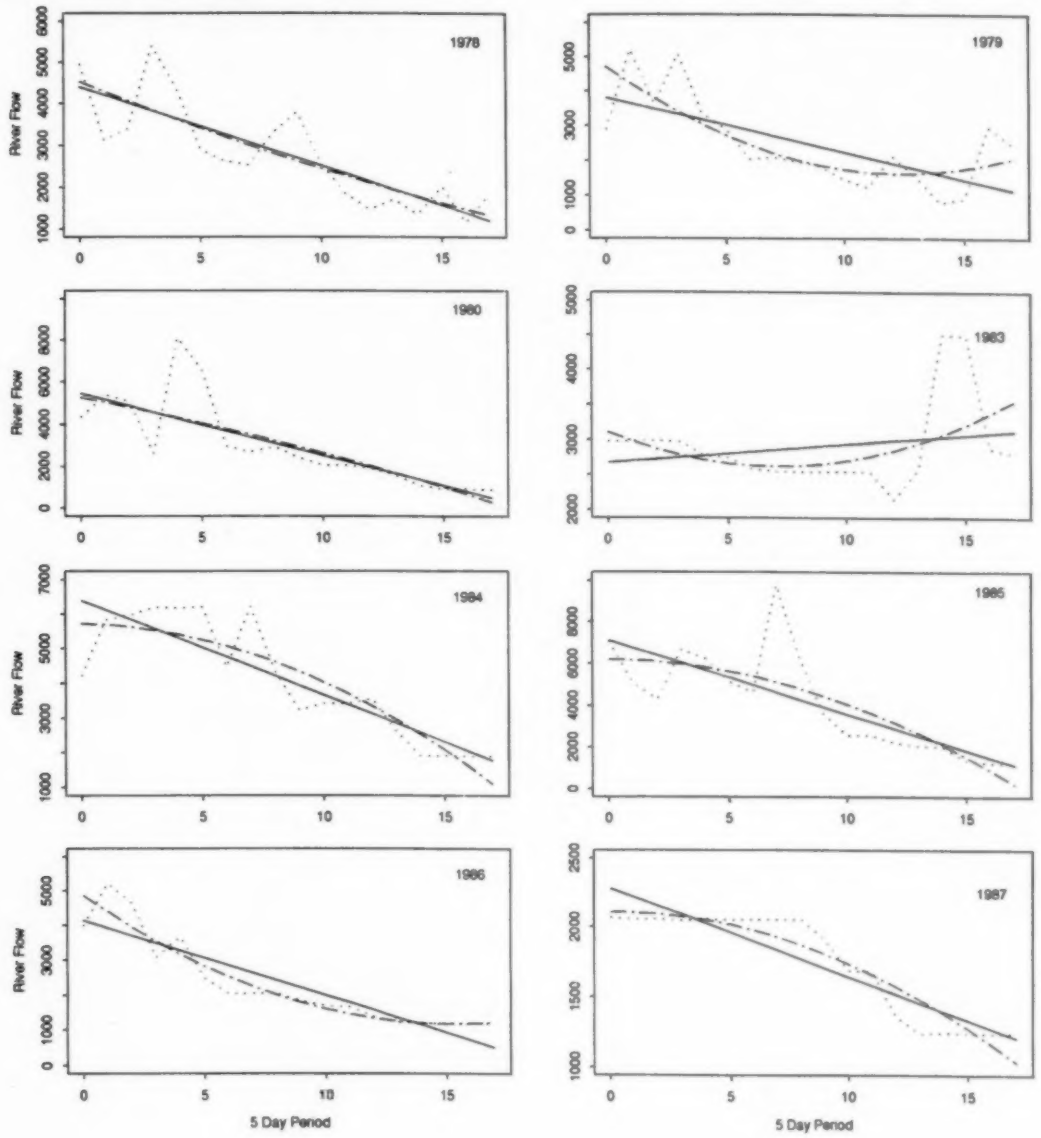
for $i = 1, \ldots, 8$. These weights (w_i) measure the relative sampling effort used in each survey.

The response model used in the weighted least squares analysis is

$$\sqrt{\hat{N}_i} = \underline{\gamma}' \underline{\beta}_i + \varepsilon_i$$

where $\underline{\beta}_i$ is the vector of independent flow variables for the ith

Fig. 1. Pattern of North Fork Lewis River flows for 1 May – 29 July in 1978–1980 and 1983–1987 based on 5-day averages (dashed lines). Linear regressions of river flow against time periods are denoted by solid lines whereas quadratic fit is denoted by dot-dashed lines.



year ($i = 1, \ldots, 8$), $\underset{\sim}{\gamma}$ is the vector of regression coefficients for the flow – smolt production relationship, and ε_i is the random error term for the ith year ($i = 1, \ldots, 8$) composed of measurement error and unexplained natural variation in smolt abundance. The WLS that is therefore minimized in the analysis of the flow – smolt production relationship is

$$[9] \qquad SS = \sum_{i=1}^{8} w_i(\sqrt{N_i} - \underset{\sim}{\gamma}'\underset{\sim}{\beta}_i)^2.$$

Approximate normality of the transformed dependent variable permits tests of significance of the regression coefficients (i.e., H_0: $\underset{\sim}{\gamma} = 0$). The objective of the WLS analysis is to estimate the regression coefficients ($\underset{\sim}{\gamma}$) associated with the flow – smolt production relationship under investigation.

In transforming the regression estimates of $\sqrt{\hat{N}}$ back to estimates of $\hat{N}$, a bias results because of the nonlinear transformation. Subsequent estimates of $\hat{N}$ from the WLS procedures were corrected for bias on the basis of a Taylor series expansion (Gemignani 1970) that took the form

$$\tilde{N}_i = \frac{\hat{N}_i}{\left(1 - \frac{\mathrm{Var}(\hat{N}_i)}{8\hat{N}_i^2}\right)}$$

$$= \frac{\hat{N}_i}{\left(1 - \frac{\mathrm{CV}(\hat{N}_i)^2}{8}\right)}$$

where CV is the coefficient of variation expressed as a fraction.

Maximum likelihood estimation

The previous WLS analysis of the $\hat{N}$ values ignores the fact that the actual random variables generated by the mark–recapture surveys are the numbers m_i, $n_{1i} - m_i$, and $n_{2i} \cdot m_i$ (or equivalently m_i, n_{1i}, and n_{2i}) that are multinomially distributed according to likelihood (eq. 1). Therefore, a more appropriate statistical analysis of the flow relationships would explicitly take into account the multinomial error structure of the capture data. This multivariate analysis can be performed by constructing a series of alternative likelihood models based on eq. 1. To test the statistical significance of the regression coefficients (i.e., H_0: $\underline{\gamma} = 0$) for the smolt abundance relationship

$$\hat{N}_i = \underline{\gamma}'\underline{\beta}_i + \varepsilon_i$$

a series of nested likelihood models (i.e., special cases of one another) must be fit using MLE. However, likelihood ratio tests will be inappropriate in testing the statistical significance of the γ values because the experimental error includes not only the multinomial measurement error associated with the surveys but also extra-likelihood variability associated with the unexplained natural variation in smolt abundance over years (Skalski et al. 1993). The above response model can be rearranged such that

$$\varepsilon_i = \hat{N}_i - \underline{\gamma}'\underline{\beta}_i$$

and where the experimental error for the ith observation is expressed as

$$\mathrm{Var}(\varepsilon_i) = \mathrm{Var}(\hat{N}_i|N_i) + \sigma^2$$

and where σ^2 is the unexplained natural variation in smolt abundance. For this reason, ANODEV must be used in tests of hypotheses concerning the abundance estimates (McCullagh and Nelder 1983, pp. 26–28). Smith (1992) used ANODEV in tests of significance when analyzing release–recapture studies of animal survival rates.

The range of alternative likelihood models for the eight-survey study includes three general types. The first model may be called the full model or saturated model in which each population survey has a unique population abundance where

$$[10] \quad L(\underline{N}, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) = \prod_{i=1}^{8} L(N_i, p_{1i}, p_{2i}|m_i, n_{1i}, n_{2i}).$$

At the other extreme, a grand mean model (Skalski et al. 1993) for the eight surveys can be constructed that has a single common value of abundance (N) for all years where

$$[11] \quad L(N, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) = \prod_{i=1}^{8} L(N, p_{1i}, p_{2i}|m_i, n_{1i}, n_{2i}).$$

Between these extremes lie the possible regression relationships of interest, where in general for the response model

$$N_i = \underline{\gamma}'\underline{\beta}_i$$

the associated likelihood model would be written as

$$[12] \quad L(\underline{\gamma}'\underline{\beta}_i, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) = \prod_{i=1}^{8} L(\underline{\gamma}'\underline{\beta}_i, p_{1i}, p_{2i}|m_i, n_{1i}, n_{2i}).$$

For example, the likelihood used to fit the population model based on the intercept term for the linear flow relationship (say β_{L_0}) would be written as

$$L(\gamma + \gamma_1\beta_{L_0}, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) = \prod_{i=1}^{8} \binom{\gamma + \gamma_1\beta_{L_0 i}}{n_{1i} - m_i, n_{2i} - m_i, m_i}$$
$$(p_{1i}q_{2i})^{n_{1i}-m_i}(q_{1i}p_{2i})^{n_{2i}-m_i}(p_{1i}p_{2i})^{m_i}(q_{1i}q_{2i})^{\gamma+\gamma_1\beta_{L_0 i}-r}.$$

Analogous likelihood models can be written for the other regression relationships investigated. The maximum likelihood estimators are asymptotically unbiased, have minimum variance, and are normally distributed (Kotz and Johnson 1985, pp. 340–346). Computer software such as FLETCH (Fletcher 1970) or EXCEL® can be used to numerically solve for the MLEs.

Essentially ANODEV tests the significance of the regression coefficients (i.e., H_0: $\underline{\gamma} = 0$) on the basis of the relative distance between likelihood extremes (eqs. 10, 11) and the proposed regression model (eq. 12). Table 3 illustrates the general structure for an ANODEV table based on the likelihoods (eqs. 10–12). The changes in deviance (i.e., likelihood ratio tests) are asymptotically chi-square distributed under the null hypotheses that the additional parameters of the more complex model have values of zero. Furthermore, the ratio of two independent chi-square random variables divided by their degrees of freedom (i.e., change in number of parameters) is F distributed. Hence, the F test in the ANODEV table is asymptotically F distributed. Skalski et al. (1993) showed the F test to be nominally distributed under a variety of multinomial tagging models. This F test incorporates the multinomial error associated with the mark–recapture surveys plus extralikelihood variation associated with lack of fit of the proposed regression model.

Furthermore, Smith (1992) showed that the quotient

$$r_D^2 = \frac{\ln L(\underline{\gamma}'\underline{\beta}_i, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) - \ln L(N, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2)}{\ln L(\underline{N}, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2) - \ln L(N, \underline{p}_1, \underline{p}_2|\underline{m}, \underline{n}_1, \underline{n}_2)}$$

is asymptotically beta distributed. The quotient (r_D^2) measures the proportion of the total deviance explained by the regression model and is analogous to the square of the multiple correlation coefficient (r^2) in standard regression analysis.

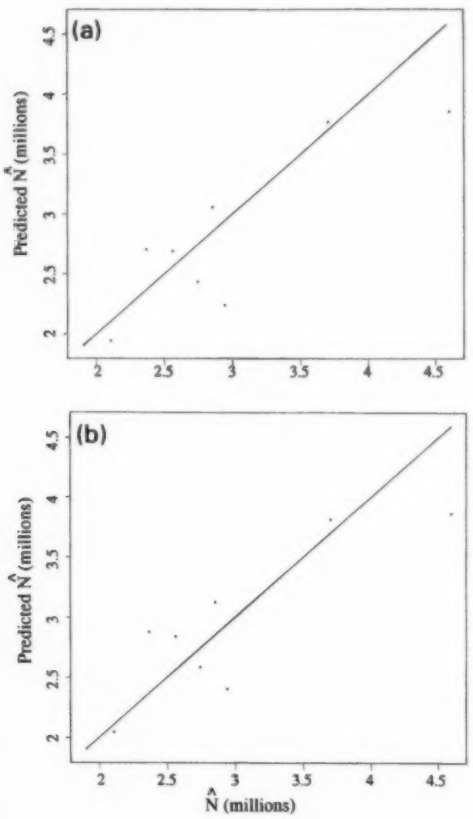
The capture parameters (i.e., p_{1i}, p_{2i}) of likelihoods (eqs. 10–12) can undergo modeling analogous to the modeling of abundance (N) described above. A chi-square test of homogeneous capture probabilities (Skalski et al. 1983) among the

Table 3. Basic structure for an analysis of deviance table (ANODEV) indicating sources of deviance, degrees of freedom, changes in deviance expressed as functions of likelihoods (eqs. 10–12), and asymptotic F test of regression model.

Source	df	Change in deviance	Dev/df	F
Total	K–1	$\text{Dev}_{\text{Tot}} = 2(\ln L(\underset{\sim}{N}) - \ln L(N))$*		
Fixed model	a	$\text{Dev}_{\text{Mod}} = 2(\ln L(\underset{\sim}{\gamma}'\underset{\sim}{\beta}) - \ln L(N))$	$\text{Mdev}_{\text{Mod}} = \dfrac{\text{Dev}_{\text{Mod}}}{a}$	$F = \dfrac{\text{Mdev}_{\text{Mod}}}{\text{Mdev}_{\text{Err}}}$
Residual	K–a–1	$\text{Dev}_{\text{Err}} = 2(\ln L(\underset{\sim}{N}) - \ln L(\underset{\sim}{\gamma}'\underset{\sim}{\beta}))$	$\text{Mdev}_{\text{Err}} = \dfrac{\text{Dev}_{\text{Err}}}{K-a-1}$	

Note: Data include K observations and the fixed model has a parameters beyond the grand mean model. $L(\underset{\sim}{N})$ is an abbreviation for the joint likelihood model $L(\underset{\sim}{N}, \underset{\sim}{p}_1, \underset{\sim}{p}_2 | \underset{\sim}{m}, \underset{\sim}{n}_1, \underset{\sim}{n}_2)$. Similar abbreviations are used for likelihood equations (11 and 12).

Fig. 2. Plots of the predicted versus observed estimates of smolt abundance for (*a*) weighted least squares analysis and (*b*) maximum likelihood estimation.



eight surveys is rejected ($P(\chi_{14}^2 > 5993.9) \approx 0$). The significant chi-square result was not unexpected because of changes in marking and recapture effort between years. Hence, the various likelihoods used in the subsequent ANODEV are based on population-specific values for p_1 and p_2 (i.e., p_{1i}, p_{2i}, $i = 1, \ldots, 8$).

Results

Weighted least squares

Weighted regression (eq. 9) was used to access the relationship between smolt abundance estimates (Table 1) and summary statistics on river flow patterns (Table 2). Simple one-variable regression models showed no significant ($p > 0.10$) relationship between flow and smolt production estimates. However, the joint regression using the intercept and slope terms in the linear river flow patterns was significant ($P(F_{[2,5]} > 4.8143) \approx 0.07$) with a multiple r^2 value of 0.6582 (Table 4). The resulting regression model was

$$[13] \quad \sqrt{\hat{N}_i} = 936.5 + 0.277\beta_{L_0,i} + 2.737\beta_{L_1,i}$$

The fitted model (eq. 13) reasonably tracks the eight abundance estimates given that flow is but one of many environmental variables influencing smolt production (Fig. 2*a*). It is important to note that both the intercept and slope terms in the linear trends in river flow were required to model production estimates. In other words, both the quantity and quality of river flows are important in modeling smolt production. The joint action of these two river flow responses appears to be important in smolt production, not the individual responses.

Multiple regression using the coefficients for the quadratic river flow patterns (Table 4) resulted in a very similar fit to the smolt production estimates ($r^2 = 0.6732$). Therefore, the linear components of flow appear to be adequate in characterizing the flow–production relationship.

Maximum likelihood estimation

MLE and tests using ANODEV resulted in relationships between river flow patterns and smolt production estimates similar to those found using WLS analysis. Both the slope and intercept parameters for the linear trend in river flows were needed to significantly ($P = 0.08$) model smolt production (Table 5). The proportion of the total deviance explained by the linear flow model is $r_D^2 = 0.6434$. Use of the coefficients from the quadratic flow model did not substantially improve the overall fit of the model ($r_D^2 = 0.6533$) and its statistical significance fell ($P = 0.20$) relative to that of the linear flow model (Table 5). The linear model fit by MLE was

$$[14] \quad N_i = 614\,416.1 + 859.3\beta_{L_0,i} + 8262.6\beta_{L_1,i}$$

Values of the ln-likelihood for alternative models fit to the

Table 4. Results of weighted least squares analysis of smolt production (Table 1) against summary variables for river flow (Table 2) for 1977–1979 and 1982–1986.

Independent variables in regression model	r^2	*P* level
β_{L_0}	0.2071	0.26
β_{L_1}	0.0291	0.69
β_{L_0}, β_{L_1}	0.6582	0.07
β_{Q_0}	0.1715	0.31
β_{Q_1}	0.1161	0.41
β_{Q_2}	0.1436	0.35
β_{Q_0}, β_{Q_1}, β_{Q_2}	0.6792	0.17

Table 5. Results of ANODEV of smolt production (Table 1) against summary variables for river flow (Table 2) for 1977–1979 and 1982–1986.

Independent variables in regression model	r_D^2	*P* level
β_{L_0}	0.3097	0.15
β_{L_1}	0.0876	0.48
β_{L_0}, β_{L_1}	0.6434	0.08
β_{Q_0}	0.2526	0.20
β_{Q_1}	0.2127	0.25
β_{Q_2}	0.2602	0.20
β_{Q_0}, β_{Q_1}, β_{Q_2}	0.6533	0.20

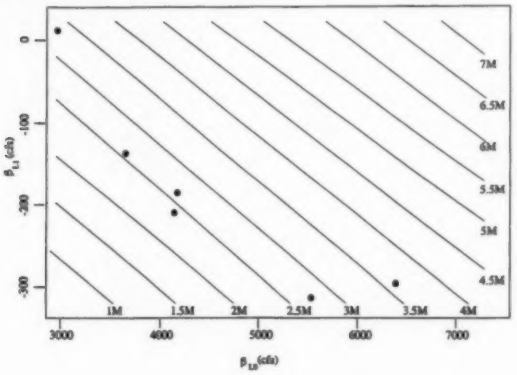
Table 6. Ln-likelihood values for alternative likelihood models fit to the capture data from eight single mark–recapture surveys of chinook smolt production (Table 1).

Model		
Description	Notation	Ln-likelihood
Full model	$L(\underset{\sim}{N_i}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−113.9136
Grand mean model	$L(N\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−130.2326
Regression β_{L_0}	$L(\gamma, \beta_{L_0}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−125.1789
Regression β_{L_1}	$L(\gamma, \beta_{L_1}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−128.8030
Regression β_{L_0}, β_{L_1}	$L(\gamma, \beta_{L_0}, \beta_{L_1}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−119.7336
Regression β_{Q_0}	$L(\gamma, \beta_{Q_0}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−126.1095
Regression β_{Q_1}	$L(\gamma, \beta_{Q_1}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−126.7620
Regression β_{Q_2}	$L(\gamma, \beta_{Q_2}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−125.9869
Regression β_{Q_0}, β_{Q_1}, β_{Q_2}	$L(\gamma, \beta_{Q_0}, \beta_{Q_1}, \beta_{Q_2}\|\underset{\sim}{m}, \underset{\sim}{n_1}, \underset{\sim}{n_2})$	−119.5713

capture data are summarized in Table 6. Analysis of deviance for the linear flow model is illustrated in Table 7. Comparison of predicted and observed estimates of smolt production based on this model (eq. 14) shows a fit that was similar to the model of eq. 13 based on WLS (Fig. 2). Similar findings of the importance of the linear trend in river flows (i.e., β_{L_0}, β_{L_1}) occurred regardless of the smoothing algorithm used in analyzing daily river flow volumes. Other smoothing algorithms investigated included use of 3- and 10-day average flows and 3- and 5-day running medians (Tukey 1977, pp. 210–214).

The fitted regression model (eq. 14) can be used to construct

Fig. 3. Predicted relationship between smolt production expressed in millions (M) of fish and levels of initial spring flow (β_{L_0}) and the rate of decline in flow per 5 days (β_{L_1}) expressed in cubic feet per second (cfs) (1 foot = 0.305 m). Dots show the limited range of actual river conditions observed in this study.



an isopleth plot of predicted smolt production as a function of initial spring flow levels (i.e., β_{L_0}) and the declines in rates of flow over season (i.e., β_{L_1}). The isopleth has the potential to serve as a management tool to regulate river flow for the benefit of both hydroelectric and salmon production. Figure 3 indicates increased smolt production under high values of both β_{L_0} and β_{L_1}. Furthermore, Fig. 3 indicates that there are a variety of environmental conditions (i.e., β_{L_0} and β_{L_1}) that are predicted to yield the same smolt production. Adaptive management would be called for to adjust rates of spring flows depending upon the magnitude of the snowmelt at the beginning of each season. Plots of actual river flow variables (Fig. 3) indicate that a relatively narrow range of conditions was observed during the study. Management decisions need to be tempered by the limited range of experiences and unlikely scenarios in certain regions of the plot (e.g., lower left to upper right diagonal).

Discussion

An alternative to WLS analysis is demonstrated for regressing environmental covariates against the results of mark–recapture studies. MLE and ANODEV procedures provide a direct approach for investigating abundance relationships within the context of tagging models. The inherent strengths of the likelihood approach include proper weighting of capture–recapture data and the flexibility to select a regression relationship dictated solely by the considerations of the biological process being investigated. Furthermore, no assumptions concerning the distribution of the abundance estimates are required; investigators rely instead on the asymptotic properties of likelihood ratio tests for drawing statistical inferences. Although the salmon smolt example consisted of a series of single mark–recapture surveys, the joint likelihood model can be composed of any of a variety of mark–recapture models. However, both the MLE and WLS analyses assume that the correct mark–recapture models have been specified. In the example presented in this paper the model specification was

Table 7. Analysis of deviance table illustrating the test of the relationship between linear river flow trends (i.e., $L\beta_0$ and $L\beta_1$) and smolt production estimates.

Source	df	Change in deviance	Mean deviance	*F* test	*P* value
Total_{cor}	7	2(–113.91958 – –130.23261) = 32.6381			
β_{L_0}, β_{L_1}	2	2(–119.73362 – –130.23261) = 20.9980	10.4990	$F_{[2,5]}$=4.5098	0.08
Error	5	2(–113.91358 – –119.73362) = 11.6401	2.3280		

Table 8. Comparison of weighted least squares (WLS) and maximum likelihood estimation (MLE) of regression relationships using tagging data.

Characteristics	WLS	MLE
Random variable	$\hat{N}$ values	Original capture data (i.e., $\underset{\sim}{m}$, $\underset{\sim}{n}_1$, $\underset{\sim}{n}_2$)
Distributional assumptions	$\hat{N}$ normally distributed under some transformation (e.g. square root)	Likelihood ratio test asymptotically chi-square
	Correct mark–recapture models specified	Correct mark–recapture models specified
Response model	Tied to data transformation	Any linear or nonlinear model deemed appropriate on the basis of biology
Weighting	Inversely proportional to estimated sampling error	Self-weighting
Test of significance	*F* test ANOVA	Asymptotic *F* test ANODEV
Mark–recapture models	Any mixture of valid models	Any mixture of valid models
Ease of analysis	Standard software packages	Numerical analysis of likelihood models

limited to the analysis of the fully parameterized (i.e., p_{1i}, p_{2i}) single mark–recapture model. The only minor drawback to the MLE approach is that standard software does not exist for data analysis. Instead, investigators must model and program the likelihoods and numerical analysis themselves. As a partial solution to the computational difficulties of MLE, special macros could be written to perform some of the computations using GLIM (Numerical Algorithms Group Inc. 1985). Extensions of the approach used by Cormack (1985) would be necessary to perform the regression analyses and correct for biases in the variance estimation (Cormack 1993).

In contrast, WLS procedures are readily available on many statistical software packages. However, the major disadvantage of the least squares analysis is that the potential need for a data transformation is coupled with a meaningful response model. In the salmon smolt example, a square-root transformation was useful in stabilizing the variance and achieving approximate normality of the data. However, the square-root transformation also dictated a curvilinear relationship with abundance (N). A curvilinear relationship between flow and smolt abundance might be most appropriate in this case, but it is an artifact of the data transformation and not a deliberate intent of the response model. The advantages and disadvantages of WLS and MLE are summarized in Table 8.

The advantage of MLE and ANODEV for analyzing capture data can be extended to the analysis of field experiments. Skalski and Robson (1992) illustrated the use of data transformations and normal theory statistics to test for effects on the abundance of mobile species. Comparable approaches can be developed using ANODEV for testing hypotheses of treatment and impact effects. However, a singular advantage of normal theory statistics is the ability to readily describe the sources of variability contributing to the experimental error and the ease of performing sample size and power calculations (Skalski and Robson 1992). In investigations of wild populations, the design of the field studies is as crucial as a valid data analysis. For this reason, the technical advantages of using MLE to assess abundance relationships must be balanced against the interpretative and analytical ease of normal theory and least squares analysis.

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Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St. Lawrence River

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Abstract: Studies on marine bivalves have shown that biological factors can significantly influence the bioaccumulation of metals by these organisms, but similar studies on freshwater mussels are virtually absent. This information is needed for the proper design of mussel monitoring programs in freshwater systems. A wide size range of specimens of *Elliptio complanata* and *Lampsilis radiata radiata* was collected from a metal-polluted site on the St. Lawrence River, and 35 males and females of each species were weighed, measured, aged, and individually analyzed for residues of 12 metals in their soft tissues. The best-fitting multiple regression models predicting metal concentrations in mussels from these variables were then determined. Such models explained a substantial proportion of the variability in the data, ranging from 17% for Se to 68% for Mn. In general, species was the most important factor, followed by age–size, growth rate, and sex, although age–size explained more of the variability than species for Cd and Hg. *Elliptio complanata* generally accumulated higher and less variable concentrations of metals; thus, this species would be the better choice for biomonitoring in the St. Lawrence River.

Résumé : Les études menées sur les bivalves marins ont montré que les facteurs biologiques peuvent avoir une influence notable sur la bioaccumulation des métaux par ces organismes, mais il n'existe pratiquement aucune étude similaire sur les moules d'eau douce. Cette information est pourtant nécessaire pour la conception de programmes de surveillance de ces bivalves dans les réseaux d'eau douce. Nous avons recueilli dans le Saint-Laurent, à un site pollué par des métaux, des spécimens de taille très variée d'*Elliptio complanata* et de *Lampsilis radiata radiata*, et nous avons pris 35 mâles et femelles de chaque espèce pour les peser, les mesurer, évaluer leur âge et doser individuellement les résidus de 12 métaux dans leurs tissus mous. Nous avons ensuite déterminé quels étaient les modèles de régression multiple qui convenaient le mieux pour prédire les concentrations de métaux dans les moules à partir de ces variables. Ces modèles expliquaient une partie substantielle de la variabilité des données, qui allait de 17% pour Se à 68% pour Mn. En général, le facteur le plus important était l'espèce, suivie par l'âge et la taille, le taux de croissance et le sexe, mais l'âge et la taille expliquaient une plus grande part de la variabilité que l'espèce pour Cd et Hg. Chez *E. complanata*, l'accumulation de métaux était en général plus forte et les concentrations moins variables; cette espèce pourrait donc être celle qui convient le mieux pour les opérations de biovigilance sur le Saint-Laurent.
[Traduit par la Rédaction]

Introduction

"Mussel watch" programs, which use concentrations of contaminants in the tissues of bivalves to indicate spatial and temporal trends in pollution, have been well established in marine and estuarine environments for many years. Bivalves meet most of the criteria normally used for selecting a biomonitor to evaluate the distribution and bioavailability of contaminants; an up-to-date list of these criteria is provided by Crawford and Luoma (1993). In particular, bivalves are sedentary, relatively hardy and insensitive to pollution, and of reasonable size, and they have high bioconcentration capacities for most organic and inorganic contaminants. Perhaps the most difficult criterion to satisfy is the need for concentrations of chemicals in a biomonitor to correlate well with levels of exposure (Johnson et al. 1993). Clearly, organisms that can

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Table 1. Combined loadings of metals to the Sorel delta area of the St. Lawrence River from industrial point sources (Quebec Iron and Titanium, Atlas Steel, and Tioxide Canada Inc.) in 1976–1977 and 1989, and loadings from the Richelieu River in 1976–1977.

	Loadings of metals ($kg \cdot d^{-1}$)		
Metal	Industries 1976–1977	Industries 1989	Richelieu River 1976–1977
Al	na	1 764	na
Cd	0.60 (3.5%)[a]	19	na
Cr	570 (61%)	513	na
Cu	148 (34%)	311	1135
Fe	29 101 (67%)	98 734	18
Hg	0.18 (19%)	0.11	na
Ni	225 (80%)	217	na
Pb	942 (97%)	44	109
Zn	923 (55%)	325	1587

Note: Data for 1976–1977 from Environnement Canada (1985); 1989 data on Quebec Iron and Titanium and Atlas Steel from Danielle Joly, Environmental Protection Service, Montréal (personal communication); 1989 data on Tioxide from Centre Saint-Laurent (1992). na, data not available.

[a]Percentage of total loadings from the 43 industries discharging to the river between Cornwall, Ontario, and Sorel, Quebec.

regulate metal concentrations in their tissues will not be suitable indicators of metal bioavailability. Bivalves are generally considered to be poor metal regulators in comparison with more highly evolved aquatic organisms such as fish and crustaceans (Bryan 1979); thus, they offer a distinct advantage as biomonitors.

Despite the extensive use of marine bivalves in biomonitoring programs, freshwater mussels have received surprisingly little attention (Phillips and Rainbow 1993). This has been attributed to a lack of species with widespread distributions, but the fact that marine shellfish are consumed by humans while freshwater species are not is surely a factor. The only group of freshwater organisms for which protocols are well established are commercial species of freshwater fish (Crawford and Luoma 1993; Phillips and Rainbow 1993), even though fish are not the ideal choice for all biomonitoring applications.

According to Thomson et al. (1984), ". . . metal uptake by organisms remains the only method available for estimating biologically available metal concentrations in natural systems . . . ." Although this is the main purpose of biomonitoring, organisms also provide a time-integrated measure of contaminant levels in the environment. Phillips and Rainbow (1993) believe the latter role to be even more critical in freshwater than marine systems, where temporal fluctuations are more extreme because of variations in river flows and the magnitude of trace metal sources. Unfortunately, organisms are also inherently variable. Studies on marine bivalves have shown that biological factors such as species, sex, age, size, reproductive cycle, and nutritional status can significantly influence the bioaccumulation of metals by these organisms. Although the literature on the use of freshwater mussels to monitor metal pollution appears extensive (see Metcalfe-Smith et al. 1992, Metcalfe-Smith 1994 and references therein), a closer examination reveals that most studies were limited to assessing the upstream–downstream influence of a metal-discharging industry on a local species. There have been few attempts to standardize beyond species and perhaps a limited size range, even though it has long been recognized that the considerable variation in metal residues among individual mussels ". . . remains an obstacle to more extensive applications" (Millington and Walker 1983).

As the demands placed on freshwater mussel monitoring programs move beyond the simple documentation of point source impacts and toward more complex applications such as (*i*) prioritization of sites for remedial action, (*ii*) detection of incremental change over time in response to pollution abatement initiatives, and (*iii*) supporting the wide-ranging objectives of large-scale ambient monitoring programs such as the U.S. Geological Survey's National Water-Quality Assessment Program (Crawford and Luoma 1993), it is apparent that the design of these programs will have to become more sophisticated. The principles learned from studies on marine bivalves may apply to freshwater species; however, this has not been confirmed to date (Crawford and Luoma 1993). This study attempts to quantify the effects of biological factors (species, sex, age, size, and growth rate) on concentrations of metals in the tissues of freshwater mussels from a metal-contaminated site on the St. Lawrence River. It therefore takes a step toward the development of protocols for the use of these organisms in biomonitoring programs and provides a basis for comparison with the marine literature.

Materials and methods

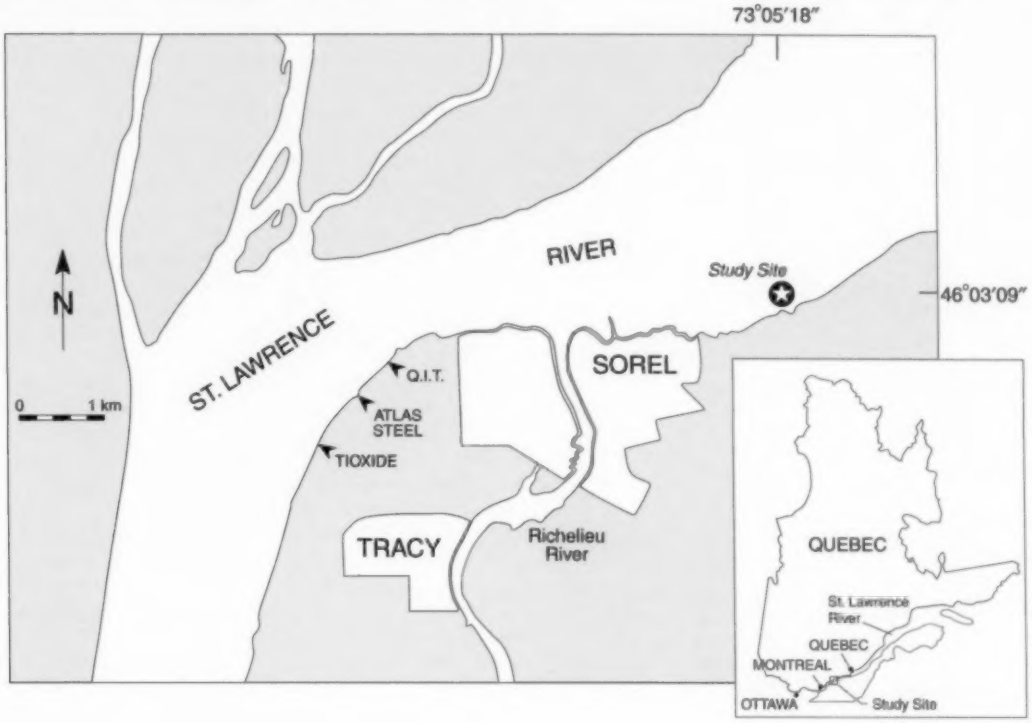
Study site

The Sorel delta area of the St. Lawrence River was chosen for this investigation because it is known to be heavily contaminated with metals. The study site (Fig. 1) was located several kilometres downstream of three major metal-discharging industries, namely, Tioxide Canada Inc., Aciers Inoxydables Atlas Inc. (Atlas Steel), and Q.I.T.-Fer et Titane Inc. (Quebec Iron and Titanium). In 1988, Tioxide produced 52 000 t of titanium dioxide pigments, Atlas Steel manufactured 60 000 t of stainless steel, and Q.I.T. produced 1 040 000 t of titanium slag (Gonthier 1991). According to a 1976–1977 survey of the 43 industries discharging into the river between Cornwall, Ontario, and Sorel, Quebec, these three industries alone contributed nearly half of the total metal loadings to this 200-km reach (Environnement Canada 1985). Loadings of Pb, Ni, Fe, Cr, and Zn from the industries, as well as Cu and Zn from the Richelieu River, were particularly high (Table 1). The most recent data available on industrial loadings are for 1989, and these are also shown in Table 1.

Collection, measurement, and ageing of mussels

In previous work (Metcalfe-Smith 1994), *Lampsilis radiata* (subfamily Lampsilinae) and *Elliptio complanata* (subfamily Ambleminae) were identified as the dominant species of unionids in the St. Lawrence River. Therefore, the study focused on these two species. A wide size range of specimens of both species was collected from the study site by divers using self-contained underwater breathing apparatus (SCUBA) on 27 June 1990 during the peak of the reproductive season, i.e.,

Fig. 1. Location of the study site on the St. Lawrence River, downstream of three major metal-discharging industries (Quebec Iron and Titanium, Atlas Steel, and Tioxide Canada, Inc.).



just prior to the release of glochidia (Clarke 1981; Trdan 1981). A total of 201 *L. r. radiata* and 134 *E. complanata* were obtained. Mussels were rinsed clean of sediment using river water, wiped dry with Kimwipes®, placed in plastic food storage bags, and immediately frozen on dry ice without being permitted to clear their digestive tracts. Several studies have shown that undigested material in the gut contains a negligible portion of the whole-body content of metals in mussels, and that gut purging may not be necessary for these organisms (see Metcalfe-Smith 1994 for a review). In the laboratory, all specimens of *E. complanata* and 125 specimens of *L. r. radiata* were thawed for 30 min, then opened, sexed by macroscopic examination of the gills, and shucked individually into acid-washed glass jars and weighed. As the latter species is dioecious and sexually dimorphic, all 201 specimens were first separated into males and females on the basis of shell shape. Then, 59 females and 66 males representing a wide size range were processed. *Elliptio complanata* cannot be sexed by external examination, and populations may include dioecious, hermaphroditic, and sequentially hermaphroditic individuals (Downing et al. 1989). In a study on Lac de l'Achigan, Quebec, Downing et al. (1989) found that hermaphrodites functioned as females but with an efficiency of ova or glochidia production that was correlated with the percentage of female tissue in their gonads. In the present study, the 65 gravid specimens were considered to be females and the 69 nongravid specimens were designated as males.

To obtain an equal number of specimens and a similar gradient of sizes for each species:sex combination, all specimens in each category were sorted into size-classes on the basis of 5-g intervals of soft tissue wet weight ranging from 5–10 to 60–65 g. Four specimens from each size-class were then arbitrarily chosen for analysis. Where fewer than four specimens were available (the under 15 g and over 50 g size-classes), all were taken. A total of 35 specimens in each category was selected and the soft tissues were individually freeze dried, weighed, and ground to a fine, homogeneous powder using a Bel-Art Micro-Mill® with stainless steel blades and grinding chamber. One male *L. r. radiata* had been misidentified as a male *E. complanata*; thus, the sample sizes were adjusted to $n = 36$ and 34 for these two categories, respectively.

The shells of all 140 specimens selected for analysis were air dried, then maximum length, height, and width were measured to the nearest 0.01 mm using vernier callipers (dimensions are illustrated in Fig. 2 of Green and Hinch 1986) and both valves were weighed together. Estimates of age for *E. complanata* and *L. r. radiata* were generally obtained by counting macroscopically visible external growth rings on the shells, which were assumed to be annual (Tevesz and Carter 1980). Shells were cleaned of sediment and attached algae, then examined using reflected and transmitted light from an incandescent lamp. The dark, annual growth rings were counted from the umbo outwards. During previous examinations of shells in this laboratory, the first visible growth ring tended to be 10–20 mm in length. This agrees with other

Table 2. Precision of age estimates for *E. complanata* and *L. r. radiata* based on the best judgement of the estimator as to the number of annual growth rings or bands that were clearly visible in the shells.

Precision of age estimate (± no. of years)	No. of specimens in each precision category			
	E. complanata		*L. r. radiata*	
	External rings	Internal bands	External rings	Internal bands
0	21	0	28	0
0.5[a]	18	0	28	0
1	17	5	12	2
1.5[b]	1	0	0	0
2	0	2	0	0
3	0	3	0	1
5–10	0	1	0	0
10–15	0	1	0	0

Note: $n = 57$ for *E. complanata* external rings, $n = 12$ for *E. complanata* internal bands, $n = 68$ for *L. r. radiata* external rings, $n = 3$ for *L. r. radiata* internal bands.

[a]Range of 1 year, e.g., 6–7.

[b]Range of 3 years, e.g., 6–9.

estimates of length at 1 year (*Lampsilis*: McCuaig and Green 1983; Day 1984; *Elliptio*: Strayer et al. 1981). Thus, for shells in which a growth ring less than 20 mm in length was not visible because of erosion of the umbo region, the first visible ring (in all cases >25 mm) was assumed to be the second year's growth ring. All specimens estimated to be less than 13 years old (49 of the 69 *E. complanata* and 60 of the 71 *L. r. radiata*) could be aged using external rings.

For approximately half of the 31 specimens aged 13 years and older (12 *E. complanata* and 3 *L. r. radiata*), counts of external growth rings were considered unreliable as estimates of age because rings were either (*i*) irregular or unusually close in their spacing (especially at the outer edges), or (*ii*) weakly differentiated by their colour and physical relief from the adjacent periostracum. In these cases, cross sections or "thin sections" of the shells (Clark 1980; Day 1984) were prepared. Valves were cut along the longest axis from the umbo to the posterior edge using a low-speed saw with a diamond blade. Cut surfaces were polished with emery and lapidary papers and glued with epoxy to microscope slides. Shells were cut a second time to leave 0.5- to 1.0-mm sections, which were polished and coated with clear nail polish. Under light microscope (dissecting and (or) compound), the dark lines extending through both the nacre and the prismatic layer were counted as annual growth bands (Day 1984).

Shell ageing is somewhat subjective; therefore, all estimates were made by the same experienced person (L.C. Grapentine). Table 2 provides a guide to the precision of age estimates based on the best judgement of the estimator as to the number of growth rings or bands that were clearly visible. Nearly 95% of the mussels in this study could be aged to within a range of 2 years. Ages of the study specimens ranged from 3 to 40 years for *E. complanata* and 2–17 years for *L. r. radiata*.

Analysis of mussels for metal residues in soft tissues

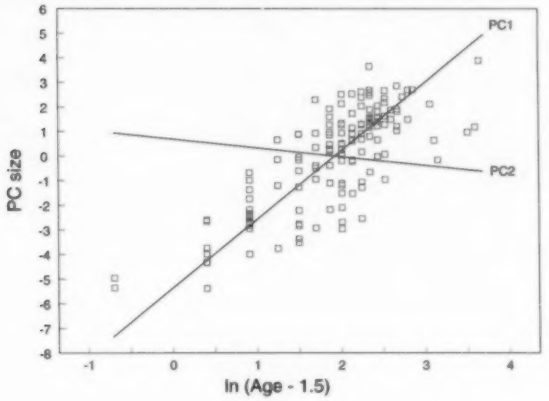
Mussels were analyzed individually for metal residues in their soft tissues by Environment Canada's National Laboratory for Environmental Testing (NLET), Burlington, Ontario, using standard procedures described in their *Manual of Analytical Methods* (National Laboratory for Environmental Testing 1994). Briefly, the analytical methods and associated detection limits (DLs) on a microgram per gram dry weight basis for the tested elements were as follows: Hg, cold vapour atomic absorption (AA) spectroscopy, DL = 0.03; As and Se, atomic emission spectroscopy using an inductively coupled argon plasma (ICAP) system, DL = 0.50 for both elements; Al, Cr, Cu, Fe, Mn, Ni, and Zn, direct aspiration AA spectroscopy, DLs = 0.50 (Ni), 2.0 (Cr, Cu, Zn), 10.0 (Fe, Mn), and 50.0 (Al); Cd and Pb, graphite furnace AA spectroscopy, DLs = 0.01 (Cd) and 0.20 (Pb). Samples were analyzed in accordance with the routine quality assurance (QA) procedures of the NLET, which include duplicate analyses to determine sample homogeneity, analysis of three reference materials to determine accuracy, spike-recovery tests to assess interference, and analysis of blanks to determine contamination owing to laboratory procedures. Samples that do not meet the QA objectives are reanalyzed, and those that still do not meet the standards are rejected. No samples from this study were rejected. However, because of insufficient material one sample could not be analyzed for As, Se, Cd, or Pb and two others could not be analyzed for Cd or Pb. Quality control reports and raw data are available from the authors.

Statistical methods

Measures of 8 biological parameters (species, sex, age, dry weight of soft tissues, and shell length, width, height, and weight) and 12 chemical parameters (concentrations of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn as micrograms per gram dry weight in the soft tissues) on 140 individual mussels from the study site constituted the data set. Linear statistical models, i.e., multivariate and univariate analysis of covariance (MANCOVA, ANCOVA), were applied to the data set to develop multiple regression models that best predicted metal concentrations in mussel tissues from biological factors. In such models relationships between variables should be linear. Therefore, the data were transformed to maximize linearity. Because interpretation of results is simpler if predictor variables are not highly correlated, principal component analysis (PCA) was used to reduce the biological parameters, which were expected to contain redundant information, to independent components prior to applying the models.

Correlation-based PCA was first performed on seven of the biological variables: species, sex, the four measures of shell size, and dry tissue weight, with the latter five variables log-transformed for allometry. The first principal component (PC1) accounted for 61% of the variability and was strongly and similarly related to all five measures of size, but unrelated to sex or species. Therefore, it was defined as PC size. PC2 accounted for 19% of the variability and represented species, with some loading on shell shape that would be species related. PC3 explained 14% of the variability and loaded entirely on sex. Because age was significantly correlated with all size variables in both species ($r = 0.48$–0.67 in *E. complanata* and 0.56–0.72 in *L. r. radiata*, $p < 0.01$), it could not be used as an independent variable in the prediction models. A further PCA was thus performed on the variables PC size and age, using the transformation ln (age – 1.5) to achieve maximum linearity.

Fig. 2. Relationship between size and age of mussels, where PC size represents all five measures of size (shell length, width, height, weight, and soft tissue dry weight) and age is ln-transformed to achieve maximum linearity with PC size. PC1 distinguishes large–old from small–young mussels and PC2 distinguishes fast-growing from slow-growing mussels.



PC1 accounted for 89% of the variability and had equal same-sign loadings on both variables. PC1 thus represented the main axis of the age–size relationship, distinguishing large–old from small–young mussels. PC2 accounted for the remainder of the variability (11%) and had equal opposite-sign loadings on the two variables. PC2 thus represented deviation from the main age–size relationship, distinguishing fast-growing from slow-growing mussels (Fig. 2).

After the eight biological parameters were reduced to four independent components, namely species (species 1 was *E. complanata*, species 2 was *L. r. radiata*), sex (sex 1 was female, sex 2 was male), age–size (PC1 of the PC size vs. transformed age PCA), and growth rate (PC2 of the PC size vs. transformed age PCA), linear models were generated using these components as the predictors and metal concentrations (ln-transformed) as the dependent variables. Covariates were the age–size and growth rate components. Initially, MANCOVAs on the full data set were used to assess significance of the four predictors across all dependent variables. Tests of significance were based on F approximations using the type III sum of squares and cross products (SSCP) matrix for the effect, and the SSCP (residual) matrix for the error terms. These were followed by a series of ANCOVAs, one for each dependent variable, to examine the influences of biological factors on the accumulation of individual metals. All effects in the univariate models (species, sex, species × sex, covariates representing age–size, and growth rate) had one degree of freedom and were tested for significance using F values calculated with the error mean square.

Results

Results of a MANCOVA test performed on the full data set showed that all biological factors were significant predictors of metal concentrations in mussel tissues (Table 3). Significance of the species × sex interaction term indicated that the influence of sex was primarily species dependent.

Table 3. Significance of biological factors as predictors of metal concentrations in mussels.

	Probability value		
		Species separate	
Predictor	Species combined	*E. complanata*	*L. r. radiata*
Species	0.0001**		
Sex	0.0947	0.0487*	0.0679
Species × sex	0.0024**		
Age–size	0.0001**	0.0001**	0.0001**
Growth rate	0.0001**	0.0013**	0.0398*

Note: Data are the results of MANCOVA tests where x represents biological factors and y represents ln-transformed concentrations of all 12 metals; $n = 137$ for species combined, $n = 69$ for *E.complanata*, and $n = 68$ for *L. r. radiata*. *, significant at $p < 0.05$; **, significant at $p < 0.01$.

MANCOVAs were also run separately for each species, and the results were similar in both cases (Table 3). Age–size was the most significant predictor, followed by growth rate, with sex being the least important factor influencing metal concentrations. Relationships appeared to be a bit stronger for *E. complanata*.

Results of ANCOVA tests showed that biological factors explained a substantial proportion of the variability in the tissue concentrations of most metals, ranging from 17% for Se to 68% for Mn (Table 4). Species was a highly significant predictor for every metal except Se and explained more of the variability than any other predictor for all remaining metals except Cd and Hg. Age–size was a significant predictor for all metals except Cr, Pb, and Se, and concentrations of Cd and Hg were much more dependent on the age–size of a mussel than its species. Growth rate was also a significant predictor for half of the metals, accounting for 2–12% of the total variability. The effect of sex was significant for only three metals (Cd, Hg, and Zn), and the species × sex interaction was significant for Cu and Se. Sex never explained more than 3% of the variability in the data.

Concentrations of Ni, Cr, Al, Cu, Pb, Hg, and Fe were significantly higher in *E. complanata* than *L. r. radiata*, whereas those of Zn, As, Mn, and Cd were significantly higher in *L. r. radiata* (Table 5). To determine whether higher concentrations were accumulated by male versus female, old versus young, or fast-growing versus slow-growing mussels, separate ANCOVAs were performed for each species and the signs of the regression coefficients (positive or negative) were used to indicate the direction of each biological effect (Table 6). Concentrations of As, Cd, Mn, Zn, Hg, and Fe were higher in older–larger individuals of both species, although the effect was not statistically significant for Fe in *L. r. radiata*. Concentrations of Cu, Al, Ni, Cr, and Se were higher in younger–smaller specimens of *L. r. radiata*, but only Cu showed this trend in *E. complanata*. Concentrations of Pb were not affected by age–size in either species. Growth rate was a significant predictor of Mn and Fe residues in both species and also of Cd, Hg, Se, and Pb in *E. complanata*. In all cases, concentrations were higher in slower growing mussels. For the three metals that had shown a significant sex effect in the full data set (Cd, Zn, and Hg), concentrations were higher in males of both species, but not significantly so. For the two metals that had shown a significant interaction between

Table 4. Significance of biological factors as predictors of metal concentrations in mussels.

	Percentage of total variability in the data explained by the model and by individual predictors (%)					
Metal	Model	Species	Age–size	Growth rate	Sex	Species × sex
Mn	68**	38**	27**	2**	—	—
Zn	59**	39**	17**	—	2*	—
Fe	55**	35**	14**	6**	—	—
Ni	51**	48**	2*	—	—	—
As	49**	38**	7**	—	—	—
Cd	49**	5**	36**	6**	2*	—
Cr	42**	41**	—	—	—	—
Pb	42**	38**	—	3*	—	—
Hg	40**	9**	25**	3*	2*	—
Al	31**	23**	6**	—	—	—
Cu	28**	17**	8**	—	—	3*
Se	17**	—	—	12**	—	3*

Note: Data are the results of ANCOVA tests where x represents biological factors and y represents ln-transformed concentrations of individual metals; $n = 137$ for Cd and Pb, $n = 139$ for As and Se, and $n = 140$ for all other metals. *, significant at $p < 0.05$; **, significant at $p < 0.01$; —, not significant.

Table 5. Comparisons of mean concentrations of metals (μg·g dry weight^{-1}) and coefficients of variation (CV) between *E. complanata* and *L. r. radiata*.

	E. complanata		*L. r. radiata*	
Metal	Mean concentration	CV (%)	Mean concentration	CV (%)
Ni	7.8**	32	3.10	88
Cr	25.8**	34	12.2	74
Al	718**	56	300	79
Cu	14.3**	25	11.3	28
Pb	10.81**	34	6.07	36
Hg	0.12**	42	0.10	40
Fe	8783**	36	5167	37
Zn	223	30	448**	58
As	3.43	22	5.43**	36
Mn	1592	48	2954**	38
Cd	0.40	70	0.51**	59
Se	2.65	16	2.58	10

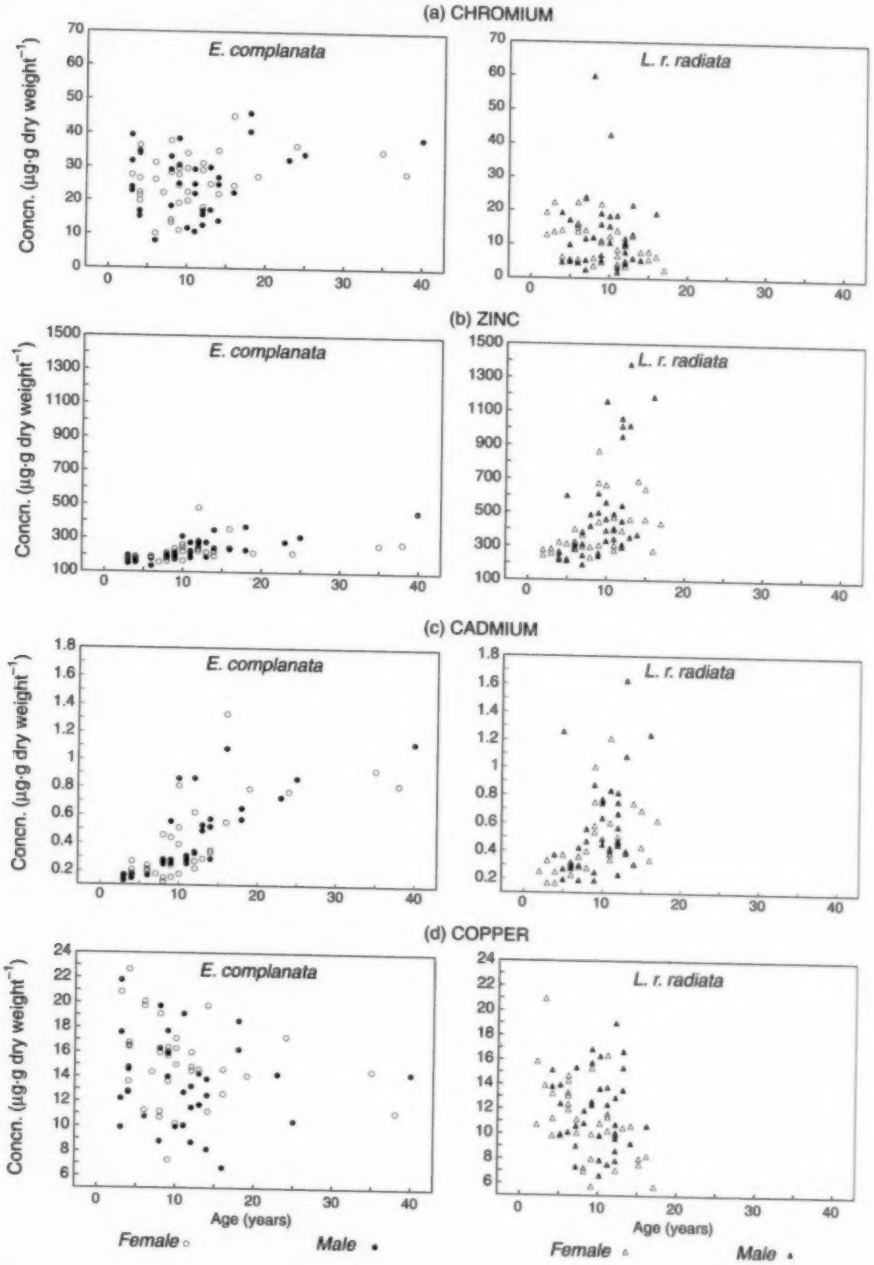
Note: CV = SD/mean × 100%; $n = 69$ for *E. complanata*, and $n = 68$ (Cd, Pb), 70 (As, Se), and 71 (other metals) for *L. r. radiata*. **, concentration significantly higher in this species at $p < 0.01$ (where variances were equal, differences between means were determined using a t test with a pooled estimate of variance and $n^1 + n^2 - 2$ df; where variances were unequal, Cochran's approximation (Snedecor and Cochran 1967) was applied).

species and sex in the full data set, Cu was higher in female *E. complanata* and Se was higher in male *L. r. radiata*.

In general, the prediction models accounted for more of the total variability in tissue metal concentrations for *E. complanata* than *L. r. radiata* (Table 6). For example, the largest proportion of variability explained for a metal in *L. r. radiata* was 29% for Cd, whereas 46–73% was explained for Cd, Fe, Hg, Mn, and Zn in *E. complanata*. The notable exception was Al, for which age–size accounted for a significant proportion of the variability in *L. r. radiata* but none of the biological factors had predictive value for *E. complanata*. On the basis of coefficients of variation, concentrations of Ni, Cr, Al, Zn, and As were much more variable in *L. r. radiata* than *E. complanata*, whereas concentrations of Mn, Cd, and Se were slightly more variable in *E. complanata* (Table 5). There were no differences in variability between species for Cu, Pb, Hg, and Fe. Thus, for most metals *E. complanata* displayed less variability among individuals, and more of it could be explained.

Although it is appropriate to use techniques such as PCA and log-transformation of the data to quantify relationships between biological factors and metal concentrations in mussels, it is helpful to return to the raw data for confirmation. Four metals selected to illustrate the various relationships are presented in Fig. 3, where concentrations of Cr, Zn, Cd, and Cu in mussel tissues are plotted against true age for each species and sex. Species accounted for 41% of total variability in the data for Cr, for which concentrations were higher in *E. complanata* than *L. r. radiata*; none of the other biological factors was a significant predictor of this metal (Fig. 3*a*). Species accounted for 39% of the total variability for Zn and concentrations were higher in *L. r. radiata* than *E. complanata* (Fig. 3*b*). Concentrations were also significantly higher in older specimens and in males of both species, but age–size explained much more of the total variability (17%) than sex (2%). Similar to Zn, levels of Cd were significantly higher in *L. r. radiata* than *E. complanata* and in older specimens and males of both species (Fig. 3*c*). However, interspecific differences accounted for only 5% of the total variability for this metal, whereas age–size accounted for 36% and growth rate (higher concentrations in slower growing mussels) an additional 6%. Copper (Fig. 3*d*) was the only metal for which concentrations were significantly higher in younger–smaller specimens of both species. The significant species × sex interaction for this metal was driven by higher concentrations in female *E. complanata*.

Fig. 3. Relationships between concentrations of metals in the soft tissues and years of age for each species and sex of mussel tested. Four representative metals are illustrated.



Discussion

Use of linear models to predict metal concentrations in bivalves from biological factors

Metal concentrations in the tissues of unionids from the Sorel delta area of the St. Lawrence River were significantly influenced by biological factors. In general, species was the most important determinant, followed by age–size, growth rate, and sex. Several other studies have used similar models to show that biological factors account for much of the variability in the metal burdens of freshwater and marine bivalves. Hinch and Stephenson (1987) analyzed the gills and bodies of

Table 6. Significance of biological factors as predictors of metal concentrations in *E. complanata* and *L. r. radiata*, the direction of each effect, and percentage of total variability in the data explained by the models.

	E. complanata				*L. r. radiata*			
	Predictor and direction of its effect			Percent variability	Predictor and direction of its effect			Percent variability
Metal	Sex	Age–size	Growth rate	explained by model (%)	Sex	Age–size	Growth rate	explained by model (%)
As	–*	+**	–	27**	+	+**	–	13*
Cd	+	+**	–**	63**	+	+**	–	29**
Mn	+*	+**	–*	73**	–	+**	–*	21**
Zn	+	+**	–	57**	+	+**	–	25**
Hg	+	+**	–**	46**	+	+**	–	23**
Fe	+	+**	–**	65**	+	+	–**	14*
Cu	–*	–*	+	12*	+	–**	+	14*
Al	–	–	+	6	+	–**	+	17**
Ni	–	–	+	5	+	–*	+	8
Cr	–	+	–	3	+	–*	+	9
Se	–	+	–**	23**	+*	–*	–	15*
Pb	+	+	–**	15*	–	–	–	6

Note: A plus sign indicates higher concentrations in male, older–larger, and faster growing mussels; a minus sign indicates higher concentrations in female, younger–smaller, and slower growing mussels. *, significant at $p < 0.05$; **, significant at $p < 0.01$.

E. complanata from two relatively uncontaminated Ontario lakes for Cd, Cu, Mn, and Zn, and found that age and shell length were frequently as important as, or even more important than, the lake of origin as sources of variability. Jones and Walker (1979) calculated multiple linear regressions of Cd, Fe, Mn, and Zn concentration against shell volume (a measure of the amount of shell material produced and thus an indicator of age) and dry body weight for the freshwater mussel *Velesunio ambiguus* from the River Murray in South Australia. Their models explained a significant proportion of the variability for all metals except Cd. Popham and D'Auria (1983) collected 20 size groups of the blue mussel, *Mytilus edulis*, from a clean and a polluted site in Burrard Inlet, British Columbia, over 13 months and determined the effects of both mussel size (dry weight) and season on concentrations of various elements in the tissues. Their models explained a significant amount of the variation for Cu, Fe, Mn, Pb, Zn, Br, and Sr. Both factors were significant determinants for most elements, although the influence of season was mainly due to seasonal changes in weight. Popham and D'Auria (1983) found that the way in which concentrations of elements were influenced by size and season differed between the two sites and concluded that regression equations derived for one location cannot necessarily be applied to another location with a different pollution status. Strong and Luoma (1981) came to similar conclusions in their study of four populations of *Macoma balthica* in San Francisco Bay.

Lobel et al. (1989) collected *Mytilus edulis* from a subtidal site in Newfoundland and determined the contribution of biological factors to the total variability in tissue residues for 25 elements. Predictors included all of those considered in the present study except age, plus condition index, various growth ratios (e.g., shell width:height), and an insolubility index that measured the contribution of gut contents and was only significant for Al. They included many redundant variables in their models and the predictive values of individual parameters were difficult to separate. However, sex was significant for the largest number of elements, followed by soft tissue dry weight and condition factor, followed by width:height ratio, which is an indicator of relative age. The importance of sex was clearly greater for *Mytilus edulis* than for *E. complanata* or *L. r. radiata* in the present study. Lobel et al. (1989) accounted for significant proportions of the variability in their data for Al, As, Cd, Cu, Mn, Pb, Se, and Zn (Cr, Fe, Hg, and Ni were not tested). In a later paper, which appears to have examined a subset of these mussels, Lobel et al. (1991) reported that chronological age was not a significant predictor of any element in specimens aged 3–14 years. They concluded that ". . . age is better dealt with in physiological terms by factors such as size, condition and growth rate . . . ." In contrast, chronological age (in combination with size) had more influence than growth rate on metal concentrations in unionids from the St. Lawrence River.

Influence of species on metal accumulation

Intuitively, one would expect species to be more important than age, size, or sex in influencing the metal concentrations accumulated by mussels from a given site. With the notable exceptions of Cd and Hg, species was the most significant predictor of metal levels in the current study. Interspecific differences observed were consistent with those reported in previous studies on the St. Lawrence and Ottawa rivers (Metcalfe-Smith 1994 and Metcalfe-Smith et al. 1992, respectively; Table 7). In most cases, these differences were so dominant that they emerged even when the samples were not controlled for other biological factors. For some elements, increasing the sample size or standardizing for biological parameters clarified the differences between species. Absolute concentrations in tissues varied between species by factors ranging from 1.2 to 2.5 times in both this study (calculated from mean values given in Table 5) and the earlier St. Lawrence River studies, and by 1.5 to 2.5 times in the Ottawa River study. Metcalfe-Smith et al. (1992) reviewed the literature comparing metal accumulation among various species of unionids and found that maximum factors of 2 to 3 times were most commonly observed. Reasons for the

Table 7. Differences in the bioaccumulation of metals by *E. complanata* (E) versus *L. r. radiata* (L) compared among several studies on the St. Lawrence and Ottawa rivers.

Metal	This study	St. Lawrence River 1989[a]	St. Lawrence River 1990[a]		Ottawa River 1985–1986[b]	
			Males	Females		
Cr	E**	E**	E*	(E)	E**	(2 of 3 sites)[c]
Ni	E**	E**	E**	E*	E*	(1 of 3 sites)
Fe	E**	E**	(E)	E*	na	
Hg	E**	E**	E**	E**	E**	(1 of 3 sites)
Al	E**	E**	E*	(E)	na	
Se	(E)	(E)	(E)	E*	E*	(2 of 3 sites)
Pb	E**	(E)	(E)	(E)	na	
Zn	L**	L**	L**	L*	L**	(3 of 3 sites)
Cu	E**	L**	(L)	(E)	L*	(2 of 3 sites)
Mn	L**	L**	(L)	L**	na	
As	L**	L*	L*	(L)	L*	(1 of 3 sites)
Cd	L**	(L)	L**	(L)	L*	(1 of 3 sites)

Note: The species with the higher concentration (E or L) and level of significance is presented. Where differences observed were not statistically significant, the species that tended to have the higher concentration is indicated in parentheses. *, significant at $p < 0.05$; **, significant at $p < 0.01$; na, not analyzed.

[a]Data for the St. Lawrence River in 1989 are results for 11 sites tested together and are composites of three males and two females per site (Metcalfe-Smith 1994). Data for the St. Lawrence River in 1990 are results for six sites tested together and are composites of five specimens per sex at each site. A paired-difference test was used to determine the mean difference between species over all sites.

[b]Data for the Ottawa River are results for three sites tested separately with 4–13 individuals per site; sex was not determined (Metcalfe-Smith et al. 1992).

[c]No difference between species at the remaining site(s).

differential uptake of metals by *E. complanata* versus *L. r. radiata* are not presently known. Both species are filter feeders and they coexist in mixed colonies, suggesting that their exposure regimes should be similar.

Influence of size and age on metal accumulation

Since the early work of Boyden (1977) identified a strong link between organism size and metal uptake in shellfish, the influence of size and, to a lesser extent, age on metal accumulation by marine and freshwater bivalves has been a topic of considerable research. The magnitude and direction of these effects have been shown to vary greatly among and within metals, species, and studies (Brix and Lyngby 1985; Hinch and Stephenson 1987; Elder and Collins 1991). As a result, few generalizations have been made. While some differences among metals and species might be expected, differences among studies on the same metals or species are more difficult to explain. Much of the confusion may be due to the great variety of conditions under which these relationships have been tested. Factors such as sample size, the biological response variables measured and their ranges (Boyden 1977; Bryan and Uysal 1978), sampling season (Strong and Luoma 1981; Lobel et al. 1991), and the pollution status of the study site (Manly and George 1977; Popham and D'Auria 1983) have considerable influence on the relationships between size or age and metal concentrations in bivalves.

Twenty-five papers on the influence of size or age on metal uptake by bivalves, including 8 on freshwater mussels and 17 on marine bivalves, were reviewed for comparison with the present study. Only studies on natural populations were considered, i.e., laboratory experiments and caged mussel studies were not included. All studies considered size (Table 8), and five also considered age. Differences in sampling protocols and environmental conditions among studies made direct comparisons difficult. However, the body of information was examined for prevailing trends. Because age and size were significantly correlated in *E. complanata* and *L. r. radiata* populations from the Sorel delta, it was assumed that the effect of age–size in the present study would be comparable to the effect of size in other studies. In fact, separate linear regressions of metal concentrations against age and size yielded the same trends. The influence of age will be specifically addressed later in this section.

Unfortunately, there have been few studies to date on freshwater mussels and most were conducted at uncontaminated sites. Furthermore, Merlini et al. (1965), Renzoni and Bacci (1976), and Hinch and Stephenson (1987) used concentrations in organs, rather than whole soft tissues, to determine relationships. In general, however, relationships between size and metal concentrations in mussels were more often significant at polluted sites (57% of 21 tests) than at clean sites (37% of 71 tests; Table 8). Where significant relationships occurred, they were usually negative at clean sites (54% of significant tests) and positive at polluted sites (58%). Trends for individual metals were unclear except that concentrations of Cu tended to be higher in smaller specimens.

The data on marine bivalves are more extensive (Table 8), but how readily they can be applied to freshwater mussels is

Table 8. Summary of the literature on relationships between organism size and concentrations of metals in the soft tissues of freshwater mussels and marine bivalves from clean and metal-polluted sites.

	Numbers of clean and polluted sites at which negative, insignificant, and positive correlations observed							
	Clean sites			Polluted sites			No. species	
Metal	Negative	Insignificant	Positive	Negative	Insignificant	Positive	tested	References
Freshwater mussels								
Cd	8	18	2	—	—	3	3	5, 10, 21, 24
Cu	2[a]	4	1[a]	3	1	—	3	5, 21, 22
Cr	—	—	1	—	—	—	1	22
Fe	2	2	1	—	—	—	2	10, 18, 22
Mn	1	2[b]	3[b]	—	—	—	3	1, 10, 21, 22
Ni	—	4	—	—	3	—	1	5
Pb	—	4	—	—	—	3	1	5
Zn	1	8[c]	4[c]	—	3	—	4	5, 10, 18, 21, 22
Hg	—	3	—	2	2	1	2	2, 5
Totals	14	45	12	5	9	7	4	
Marine bivalves								
Cd	3	5	2	3	4	4	10	3, 4, 6, 7, 9, 11, 14, 15, 17, 20, 25
Cu	9[d]	4	3[d]	10[e]	2	3[e]	12	3, 4, 6, 7, 9, 11, 13, 14, 15, 17, 19, 20, 25
Cr	2	1	—	—	1	1	3	3, 6, 7, 20
Fe	12	2	—	5	3	—	10	3, 4, 6, 7, 9, 11, 19, 23
Mn	5	4	—	5	2	—	7	3, 4, 6, 7, 9, 11, 15, 25
Ni	3	2	1	4	2	2	8	3, 4, 6, 7, 11, 14, 15
Pb	3	5	1	3	2	4	11	3, 4, 6, 7, 9, 14, 17, 19, 20, 25
Zn	7	9	1	1	3	4	11	3, 4, 6, 7, 9, 11, 14, 15, 16, 17, 19, 20, 25
Hg	—	—	—	—	—	2	1	8, 20
Al	1	—	—	—	—	—	1	25
As	1	1	—	—	1	1	2	12, 25
Se	—	1	—	—	—	—	1	25
Totals	46	34	8	31	20	21	14	

Note: Size parameters tested included dry weight (15 studies), wet weight (3 studies), and shell length (7 studies). References: 1, Merlini et al. 1965; 2, Renzoni and Bacci 1976; 3, Watling and Watling 1976; 4, Boyden 1977; 5, Manly and George 1977; 6, Bryan and Hummerstone 1978; 7, Bryan and Uysal 1978; 8, Davies and Pirie 1978; 9, Harris et al. 1979; 10, Jones and Walker 1979; 11, Cossa et al. 1980; 12, Langston 1980; 13, Strong and Luoma 1981; 14, Klumpp and Burdon-Jones 1982; 15, Latouche and Mix 1982; 16, Lobel and Wright 1982; 17, Ritz et al. 1982; 18, Millington and Walker 1983; 19, Popham and D'Auria 1983; 20, Brix and Lyngby 1985; 21, Hinch and Stephenson 1987; 22, Baudo and Galanti 1988; 23, Brown and Kumar 1990; 24, Campbell and Evans 1991; 25, Lobel et al. 1991.

[a]At one site, gills showed a negative correlation and bodies a positive correlation.

[b]At one site, gills showed no relationship and bodies showed a positive correlation.

[c]At two sites, gills showed no relationship and bodies showed a positive correlation.

[d]At two sites, correlations changed from negative to positive depending on season.

[e]At one site, correlations changed from negative to positive depending on season.

not known. For example, many of the species tested were deposit feeders that might be expected to behave differently than filter feeders. As for freshwater mussels, however, relationships between size and metal concentrations in mussels were more often significant at polluted sites (72% of 72 tests) than at clean sites (61% of 88 tests). In general, significant relationships were more common among marine than freshwater bivalves. Where significant relationships occurred, they were usually negative at both clean (85% of significant tests) and polluted (60%) sites. However, positive relationships were observed more frequently at polluted sites. This suggests that at relatively uncontaminated sites, bivalves are able to regulate at least some metals such that body burdens do not accumulate over time. However, at polluted sites these mechanisms fail and body burdens increase with size and age.

Trends for marine bivalves were examined on a metal by metal basis and were in several cases similar to those observed for freshwater mussels. Concentrations of Cu in marine and freshwater bivalves, including *E. complanata* and *L. r. radiata*, were consistently higher in smaller animals. Cu is an essential nutrient that may be well regulated by many species, thus preventing concentrations from increasing with age. Zn

demonstrated the same trend as Cu in marine bivalves from clean sites, but the reverse trend at polluted sites.Concentrations of Zn were also higher in older–larger unionids from the Sorel delta area, which is known to be highly contaminated with Zn. This suggests that the regulatory capabilities of bivalves for Zn, which is also an essential nutrient, may be more limited than those for Cu. Concentrations of Ni were usually higher in smaller marine bivalves, and this was also observed for unionids. Fe and Mn were higher in smaller marine bivalves at all sites where significant relationships were observed. In contrast, Fe and Mn were higher in larger *E. complanata* and *L. r. radiata* in the present study. Seah and Hobden (1969) and Hobden (1970) found that Mn and Fe were actively accumulated by *E. complanata* and stored in an insoluble form that was not depleted after 6 months of starvation. In earlier work, they had found that concentrations of Fe in *Mytilus edulis*, which were initially one third of those in *E. complanata*, decreased steadily under conditions of starvation until a stable level of permanently stored Fe was reached. It would appear that unionids have a greater capacity for storing Fe and Mn, which would accumulate over time and hence be higher in larger animals. Trends for Pb and Cd were variable, with negative, positive, and insignificant effects observed in marine bivalves from both clean and polluted sites. Age–size did not influence Pb concentrations in either *E. complanata* or *L. r. radiata*, but Cd levels were higher in older–larger specimens of both species. Data on Al, As, Cr, Hg, and Se were insufficient to draw any general conclusions from the marine data, although the two studies on Hg reported, as we did, higher concentrations in larger animals.

For the six metals showing an increase in concentration with increasing age–size in *E. complanata* and *L. r. radiata*, this effect explained a considerable proportion of the variability in the data for all metals except As. For the five metals showing an increase in concentration with decreasing age–size in one or both species, the proportion of the variability accounted for was much lower. According to Strong and Luoma (1981), smaller individuals of many bivalve species accumulate higher concentrations than larger individuals because of their more rapid uptake rates. For metals that do not accumulate over time, i.e., those that can be regulated, a negative correlation may occur when young animals have been included in the sample. For slow-exchanging metals such as Cd and Hg that have been shown to accumulate with age (Strong and Luoma 1981), the negative influence of younger animals may be offset and an overall positive correlation for the metal would result. Negative correlations between age–size and metal levels were significant for Cu, Al, Ni, Cr, and Se in *L. r. radiata*, but only for Cu in *E. complanata*. Metcalfe-Smith (1994) found that *L. r. radiata* displayed a narrower range of tissue concentrations for all metals except Mn and Zn than sympatric *E. complanata* from 11 sites of varying pollution status on the St. Lawrence River, and concluded that *L. r. radiata* may be more capable of regulating metals. This would be consistent with the greater number of negative correlations observed for this species in the present study.

Bivalves tend to become larger as they age, hence one would expect age and size to be directly related and the influence of both factors on the accumulation of metals to be the same. Many studies on unionids have demonstrated significant positive correlations between age and various measures of size (see Metcalfe-Smith and Green 1992 for a review). However, others have found age and size to be poorly related (e.g., Hinch and Stephenson 1987; Hanson et al. 1988). As previously mentioned, only five investigators considered the influence of both size and age on the accumulation of metals by bivalves. Of these, only two aged their specimens, as we did, by counting annual growth bands in the shells (Hinch and Stephenson (1987) for *E. complanata* and Lobel et al. (1991) for *M. edulis*). Langston (1980) inferred ages of *Scrobicularia plana* from shell length using the Walford Plot method, while Jones and Walker (1979) and Millington and Walker (1983) used shell volume as a surrogate for age in their studies on *Velesunio ambiguus*.

One might expect that where age and size were correlated, the two factors would have the same effect on metal uptake. Conversely, where age and size were not correlated, their effects might differ. This was generally true, although there were exceptions. Langston (1980) found that age and size of *S. plana* were correlated in three different populations, and that the effects of the two parameters on As concentration were the same, i.e., effects of both age and size were negative in an uncontaminated estuary, positive in a polluted estuary, and insignificant at an intermediate site. Jones and Walker (1979) reported that age and size were not correlated in *V. ambiguus* from a site on the River Murray, and that concentrations of Fe, Mn, and Zn increased with age but decreased with dry weight. Millington and Walker (1983) sampled the same site in the same year and confirmed the result for Fe, although they reported strong correlations between age and size for this population. Hinch and Stephenson (1987) determined that age and size were not correlated in populations of *E. complanata* from Beech and Tock lakes in Ontario. However, the effects of age and size differed significantly in only 1 of 16 comparisons involving four metals and two components of the soft tissues: concentrations of Zn increased with size but decreased with age in the visceral mass of mussels from Beech Lake. Finally, Lobel et al. (1991) examined the effects of age and size on concentrations of 24 elements in *Mytilus edulis*. The effect of size (dry weight) was significant for 14 elements and negative for all except K. Chronological age was not a significant predictor of any element, although width to height ratio (W:H), which is an indicator of relative age, was significant for seven elements and always positive. Lobel et al. (1991) did not state whether age and size were correlated in their study population, but it appears that they were not. The use of W:H as an indicator of age deserves comment. Animals age at rates that are individually determined by genetic and environmental factors. A parameter such as W:H, which represents the physiological age of an individual, should therefore be a more sensitive indicator of the ageing process than a categorical parameter such as chronological age. This might explain why significant relationships were observed for W:H but not for years of age in Lobel et al.'s (1991) study. In the present study, concentrations of As, Cd, Fe, Hg, Mn, and Zn in *E. complanata* and *L. r. radiata* were found to be higher in older specimens. The same relationship was also observed for these metals in several other studies, e.g., Fe, Mn, and Zn in *V. ambiguus* (Jones and Walker 1979; Millington and Walker 1983), As and Cd in *Mytilus edulis* (Lobel et al. 1991), and As in *S. plana* from a contaminated site (Langston 1980).

Table 9. Ranges of metal concentrations (μg·g dry weight^{-1}) in *E. complanata* and *L. r. radiata* from the Sorel study site versus ranges in mussels from 11 sites of differing pollution status (including the Sorel site) on the St. Lawrence River.

Metal	Concentration range and magnitude of difference between lowest and highest value			
	E. complanata		*L. r. radiata*	
	All 11 sites[a] (composite samples)	Sorel site only (individual mussels)	All 11 sites[a] (composite samples)	Sorel site only (individual mussels)
Al	153–696 (5×)	<50–1760 (35×)	146–534 (4×)	<50–901 (18×)
As	2.8–7.5 (3×)	2.1–4.5 (2×)	4.2–8.6 (2×)	2.7–10.4 (4×)
Cd	0.4–7.1 (18×)	0.1–1.1 (11×)	0.6–8.3 (14×)	0.2–1.6 (8×)
Cr	3.3–18.8 (6×)	8.1–45.9 (6×)	2.0–12.6 (6×)	2.0–60.0 (30×)
Cu	7.8–13.6 (2×)	6.7–22.7 (3×)	8.7–16.2 (2×)	5.5–20.7 (4×)
Fe	1140–9980 (9×)	3640 – 16 220 (4×)	771–7120 (9×)	2040 – 11 700 (6×)
Hg	0.07–0.33 (5×)	0.07–0.28 (4×)	0.05–0.13 (3×)	0.06–0.31 (5×)
Mn	1430–4690 (3×)	593–3510 (6×)	1740–7380 (4×)	1330–6030 (5×)
Ni	2.1–7.0 (3×)	2.2–13.1 (6×)	1.2–3.9 (3×)	0.5–19.9 (40×)
Pb	0.9–13.2 (15×)	5.2–18.8 (4×)	0.7–7.5 (11×)	1.6–13.2 (8×)
Se	2.0–5.2 (3×)	2.0–3.2 (2×)	2.0–3.0 (2×)	1.9–3.1 (2×)
Zn	130–280 (2×)	136–312 (2×)	190–511 (3×)	196–1380 (7×)

Note: Comparisons are for specimens of both sexes collected in 1990 and measuring 6.0–10.0 cm in shell length.
[a]Data from Metcalfe-Smith (1994).

Influence of growth rate on metal accumulation

By using PCA, we were able to extract a growth rate component from the data set and to evaluate its influence on metal concentrations separately from age and size per se. The effect of growth rate was significant for Cd, Fe, Hg, Mn, Pb, and Se in *E. complanata*, but only for Fe and Mn in *L. r. radiata*. In all cases, concentrations were higher in slower growing mussels. The effect may have been more pronounced in *E. complanata* because of the wider range of ages, and thus growth rates, among the tested specimens. Over the full range of specimens examined, the average yearly increment in soft tissue dry weight was 0.09 g for *E. complanata* versus 0.23 g for *L. r. radiata*. These values are not directly comparable, because all *L. r. radiata* were 17 years old or younger, whereas nine *E. complanata* were between the ages of 18 and 40. However, when growth rates of specimens aged 3–17 years were compared, the average yearly increment was still 20% lower for *E. complanata* (0.20 g) than *L. r. radiata* (0.24 g).

Other investigators have consistently reported higher concentrations of metals in slower growing bivalves whenever the effect of growth rate was significant. Davies and Pirie (1978) examined trends for Hg in separate size-classes of *Mytilus edulis* from the Firth of Forth, Scotland, and in the population as a whole. They found that concentrations of Hg increased with increasing wet tissue weight at the population level, but within each size-class the correlation tended to be negative. Williamson (1980) made the same observation in a study of Cd accumulation by the land snail *Cepaea hortensis*. Langston (1980) found that the growth rate (increase in dry soft tissue weight with age) of *S. plana* slowed with age in a contaminated estuary in Wales and accelerated with age in a clean estuary. In both locations, concentrations of As were higher in slower growing clams; thus, levels increased with age at the contaminated site and decreased with age at the clean site. Lobel and Wright (1982) determined the influence of various biological factors on levels of Zn in *Mytilus edulis* from the Tyne estuary, United Kingdom, and used the ratio of soft tissue dry weight to shell dry weight as an indicator of flesh condition (FC). Although not strictly comparable to the growth rate parameter used in the present study, both variables provide a measure of the degree of dilution by soft tissue. Lobel and Wright (1982) found that FC was negatively correlated with Zn concentration, suggesting that levels were higher in animals that were "less meaty." In later studies on *Mytilus edulis* from Newfoundland, Lobel et al. (1991) replaced FC with CI (condition index = soft tissue dry weight : (shell length × width × height)), because they felt it was a more reliable indicator of condition. They concluded that CI was the most important variable influencing element concentrations in mussels, because it was highly significant for all chemical classes. For 14 of the 24 elements tested, including Cd, Cu, Mn, and Pb, concentrations were higher in specimens with a low flesh weight for the size of their shell. Concentrations of Al, As, Se, and Zn were unaffected.

Influence of sex on metal accumulation

Sex was the least important factor influencing metal concentrations in unionids, accounting for at most 3% of the overall variability in the data. Concentrations of Cd, Hg, and Zn were higher in males of both species, but the effect appeared to be weak because it only reached statistical significance when all 140 specimens were considered. The effect of sex on levels of As, Cu, Mn, and Se was species dependent, i.e., concentrations differed between the sexes for one species but not the other. In a related study that was conducted concurrently, Metcalfe-Smith (1994) compared metal concentrations in males and females of these species from six sites on the St. Lawrence River. Samples were analyzed as composites, and differences between the sexes were determined over all sites using a paired-difference test. The Sorel study site was included in the investigation. Concentrations of Cu were found to be higher in female than male *E. complanata*, while concentrations of Cd,

Fe, Se, and Zn were higher in male than female *L. r. radiata*. The results of the two studies taken together suggest that Cu and As tend to be higher in female mussels, while Cd, Fe, Hg, Mn, Se, and Zn tend to be higher in males. There were no apparent differences between the sexes for Al, Cr, Ni, or Pb.

Studies on marine mussels have shown that concentrations of metals in both males and females are highest immediately prior to spawning, and that differences between the sexes are at a minimum during this period (see Metcalfe-Smith 1994 for a review). In this study, unionids were collected just before releasing their glochidia. Thus, it is possible that sex may be a more important source of variability at other times of the year. Only one other study on a freshwater mussel was available for comparison. Jones and Walker (1979) found no differences in the accumulation of Cd, Fe, Mn, or Zn by male versus female *V. ambiguus* but did not describe the reproductive status of the specimens. The literature on marine bivalves generally showed that sex was an important predictor of metal residues and, in contrast to the results for unionids, that levels were usually higher in females. The results of five studies (Watling and Watling 1976 for *Choromytilus meridionalis*, no season given; Orren et al. 1980 for postspawn *C. meridionalis*; Klumpp and Burdon-Jones 1982 for prespawn *Trichomya hirsuta*; Latouche and Mix 1982 for *Mytilus edulis*, no season given; Lobel et al. 1989 for postspawn *Mytilus edulis*) were remarkably consistent. They indicated that Cu, Fe, Mn, Zn, and probably also As and Se were higher in female mussels, Pb was higher in males, and there were no differences between the sexes for Cd, Ni, and probably also Al and Cr. Lobel et al. (1991) determined that sex explained most of the variability in their data for As, Cu, Mn, Se, and Zn in *Mytilus edulis*.

Biological versus environmental variation

Biological factors explained a significant proportion of the variation in metal concentrations among mussels from the study site at Sorel, Quebec. Nevertheless, standardization for biological factors in mussel monitoring programs would not be worthwhile if within-site variation were negligible relative to variation in metal concentrations among mussels from sites of differing pollution status. Comparisons between the ranges of metal concentrations observed in individual mussels from the study site and those in composite samples of mussels from 11 sites throughout the St. Lawrence River (Table 9) show that the amount of variation arising from within-site (largely biological) versus among-site (largely environmental) sources is indeed significant. Within-site variation was similar to among-site variation for As, Cu, Hg, and Se in both species, Cr and Zn in *E. complanata*, and Mn in *L. r. radiata*. Within-site variation was lower than among-site variation for Cd, Fe, and Pb in both species, but was actually higher for Al and Ni in both species, Mn in *E. complanata*, and Cr and Zn in *L. r. radiata*.

Conclusion

In conclusion, biological factors significantly influenced the concentrations of metals accumulated by freshwater mussels from a metal-contaminated site on the St. Lawrence River. Standardizing for these factors, or accounting for them in multiple regression models, would therefore greatly improve precision in biomonitoring programs that use mussels to determine spatial and temporal trends in metal pollution. Species, size, age, and probably growth rate should all be considered when designing a mussel monitoring program, but sex could be ignored at little cost. The influences of biological factors sometimes differed greatly among metals; however, relationships for a given metal were usually similar in both species. *Elliptio complanata* would be the better choice for biomonitoring in the St. Lawrence River for the following reasons: (*i*) it exhibited less individual variation in metal levels than *L. r. radiata*, and more of this variation could be explained; (*ii*) it accumulated higher concentrations of most metals; and (*iii*) it was shown in earlier work (Metcalfe-Smith 1994) to have a broader range of responses than *L. r. radiata* to the same exposures, suggesting that it may be more sensitive to changes in pollution status. Comparisons between this study and the marine literature revealed many inconsistencies with respect to the relative importance of various biological factors as predictors for certain metals and the magnitude and direction of their effects on tissue concentrations. For example, sex seemed to be more important and age less important in marine bivalves, and the influence of size was usually significant regardless of the pollution status of the study site. There may be fundamental differences in the mechanisms of bioaccumulation between marine and freshwater mussels or in the behaviour of metals in marine versus freshwater systems. However, more studies must be conducted in freshwater systems before we can determine if marine mussel watch protocols are applicable to freshwater mussel monitoring programs, or if new protocols must be developed.

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Growth and migration of 0-year American shad (*Alosa sapidissima*) in the Hudson River estuary: otolith microstructural analysis

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Abstract: A fine-scale demographic study of young-of-year American shad (*Alosa sapidissima*) was undertaken in May–November 1990 using otolith microanalysis (N = 280 larvae and 1507 juveniles) to elucidate patterns of growth and seaward migration. Migration was both size- and age-biased. Upriver fish were consistently smaller and younger than downriver fish until late in the season. However, cohorts moved down the river as early as June, older cohorts tending to move earlier than younger ones. Maximum relative condition was attained at the up- and mid-river sites in early August and declined steadily thereafter; maximum values were high in mid- to late July in the downriver collections and declined in the fall. The year-class was established mainly by cohorts hatched after 1 June, whereas most spawning activity occurred in early to mid-May. This was probably due to (*i*) a severe weather event in mid-May that caused high river flows and low temperatures, thereby reducing available food and resulting in high larval mortality, and (*ii*) rapid warming in June and low river flows, allowing food sources to build up and promoting larval survival.

Résumé : Une étude à petite échelle de la démographie de jeunes de l'année d'alose savoureuse (*Alosa sapidissima*) a été menée de mai à novembre 1990 à l'aide de la microanalyse des otolithes (N = 280 larves et 1507 juvéniles); elle visait à faire la lumière sur les schémas de croissance et de migration vers la mer. La migration présentait un biais à la fois selon la taille et selon l'âge. Les poissons d'amont étaient régulièrement plus petits et plus jeunes que les poissons d'aval jusque tard dans la saison. Toutefois, les cohortes descendaient la rivière dès le mois de juin, les cohortes plus âgées dévalant plus tôt que les plus jeunes. La condition relative maximale était atteinte aux sites d'amont et de mi-rivière au début d'août, puis baissait régulièrement par la suite; les valeurs maximales étaient élevées du milieu à la fin de juillet dans les échantillons pris en aval et baissaient à l'automne. La classe d'âge était constituée principalement par les cohortes écloses après le 1er juin, tandis que la plus grande partie de l'activité de ponte se produisait du début au milieu de mai. Ce phénomène est probablement dû (*i*) à un épisode météorologique défavorable à la mi-mai qui a causé de forts débits et des températures basses, ce qui a réduit la nourriture disponible et a causé une forte mortalité des larves et (*ii*) à un réchauffement rapide en juin et à de faibles débits, ce qui a permis aux sources de nourriture de se reconstituer et a favorisé la survie des larves.
[Traduit par la Rédaction]

Introduction

The importance of body size in the ecology of organisms has received considerable recognition in recent years and research has yielded rich insights about ecological interactions and life-history strategies (for reviews see Peters 1983; Schmidt-Nielsen 1984; Werner and Gilliam 1984; Roff 1992). In the case of anadromous fishes, the decision about when juveniles should leave natal rivers can be predicated on several factors, including size (Wedemeyer et al. 1980). Achievement of some minimum size has at least two advantages: ability to avoid many gape-limited predators (e.g., Poe et al. 1991) and accumulation of sufficient energy stores for long-distance travel (Taylor 1990*a*).

For several species of anadromous salmonids (the most widely studied migrating fish), juvenile out-migration is size biased (Hoar 1976; Wedemeyer et al. 1980; Jonsson et al. 1990; Taylor 1990*b*; Ivanter 1991; Svenning et al. 1992), with larger individuals entering the smolt stage (physiologically capable of entering seawater) and leaving the nursery river before smaller ones. Higher growth rates in fish that migrate early have been documented in some cases (Taylor 1990*b*; Ivanter 1991; Svenning et al. 1992). Although the literature on nonsalmonids is sparser, size-biased juvenile migration has also been observed in roach (*Rutilus rutilus*, Pavlov et al. 1990), Atlantic croaker (*Micropogon undulatus*, Yakupzack et al. 1977), and Gulf menhaden (*Brevoortia patronus*, Deegan 1990).

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Fig. 1. Total lengths (mm) of juvenile Hudson River American shad versus distance from the river mouth, for different sampling weeks from mid-July through early November, 1986. Best-fit lines are included to aid in visualizing trends. Data source: Hudson River Fisheries Data Base, maintained by Consolidated Edison of New York, Inc.

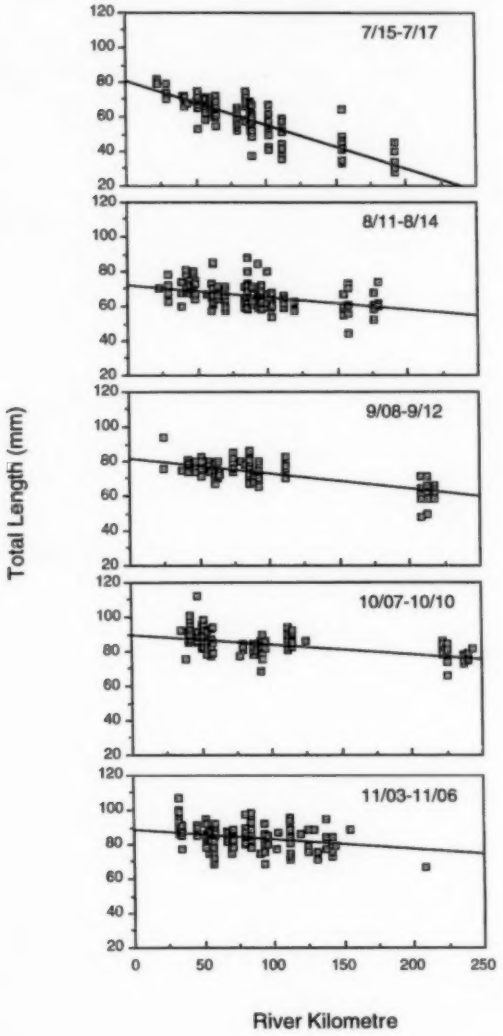
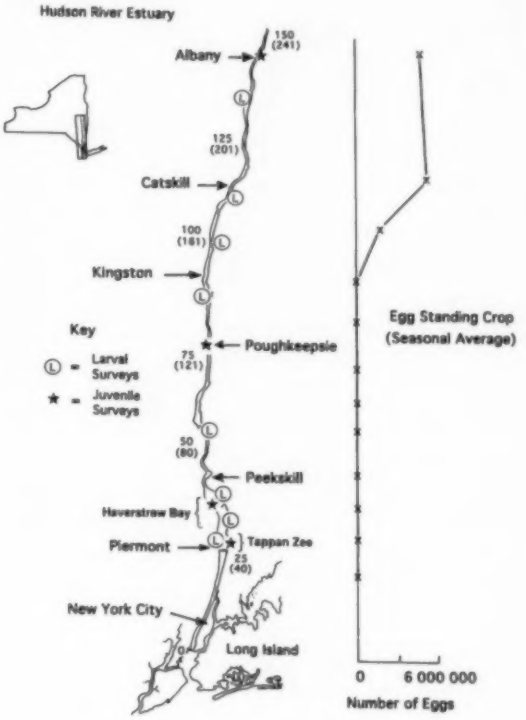


Fig. 2. Map of the Hudson River, showing sampling sites. Longitudinal distributions of American shad eggs in 1990 are shown to the right (data source: Lawler, Matusky and Skelly Engineers 1992). Distances are given in river miles and river kilometres (parentheses).



The American shad (*Alosa sapidissima*) is a large, anadromous clupeid with a geographic distribution from New Brunswick, Canada, to northern Florida, U.S.A. (Bigelow and Schroeder 1953). Because this species uses large rivers as spawning and nursery grounds, it is not easy to identify migrating juveniles. Consequently, patterns of juvenile outmigration are not clearly understood or agreed upon. In the Connecticut River, Leggett (1976) and O'Leary and Kynard (1986) observed that seaward migration occurs when temperatures drop below 19°C in the fall. In contrast, Marcy (1976) and Chittenden (1969), noting larger individuals in midsummer in downstream reaches of the Connecticut and Delaware rivers, respectively, suggested that these individuals had already begun their migration. Thus, at the population level, shad migration has been viewed either as a temperature-cued pulse phenomenon or as a gradual process that may be cued by size.

Since 1974, juvenile fish populations of a dozen species including American shad have been monitored in the Hudson River in New York state (see Klauda et al. 1988 for a description of the data sets). When fish length is plotted as a function of distance from the river mouth for different sampling dates, a spatiotemporal pattern is evident (Fig. 1): larger fish are seen downriver early in the summer, but as the season progresses, the upstream–downstream size differential diminishes. The pattern can be interpreted in two ways: (*i*) shad are spatially distributed as stationary subpopulations, with downriver shad growing much more quickly than upriver shad early in the season and then dramatically slowing in growth while growth rates accelerate upriver, or (*ii*) the downriver fish represent seaward migrants, with migration based on attainment of some minimum size. Virtually all spawning activity takes place above river kilometre (km) 160 (river mile (RM) 100) as measured from the Battery at the southern tip of Manhattan Island (Talbot 1954; Schmidt et al. 1988), as shown in Fig. 2. Thus,

fish located at downriver sites have likely moved there from upriver. The questions then become the following: how do fish migrate from upriver sites to downriver sites, and what combination of cues (intrinsic, extrinsic) affects the decision to migrate?

The purpose of the present study is to elucidate the patterns of age, growth, and movement of young-of-year (YOY) American shad in the Hudson River. I used the method of analysis of otolith microstructure (Pannella 1971; Brothers et al. 1976; Campana and Nielsen 1985) to assess daily ages and growth rates of individual fish in the 1990 year-class. With information from such analyses, and ancillary data, the following hypotheses about migration could be evaluated: (*i*) juvenile shad migration is based on age; (*ii*) migration is based on attainment of some minimum size; or (*iii*) shad migration is cued on declining fall temperatures. If the first hypothesis is true, then older fish would be observed downriver prior to younger ones; if the second holds, then within a given age cohort, larger individuals should appear downriver first. Note that age and size might be correlated. If they are, then it will be difficult to discriminate between them as cues. If the third hypothesis is true, then fish would not be expected in the lower river until that temperature occurs (after reaching the summer maximum); for a working hypothesis, the temperature cited as a cue in the Connecticut River (19°C, O'Leary and Kynard 1986) can be used as the threshold.

Materials and methods

Study area

The Hudson River, located in eastern New York state, arises in the Adirondack Mountains and flows in a southerly direction for 507 km (315 RM), draining into New York Bay south of Manhattan. A dam located at Green Island (km 245, RM 152) effectively separates the lower, tidal portion from the nontidal upper river. American shad now use the tidal portion of the Hudson exclusively; prior to the construction of the Green Island Dam, spawning extended up to Baker's Falls (km 314, RM 195; Atlantic States Marine Fisheries Commission 1985).

Larval collections

Larval American shad were collected with a 3-m beach seine (0.32-cm mesh). Seven sites (Fig. 2) ranging from km 40 to km 218 (RM 25–135) were sampled every 2 weeks between 19 May and 23 June 1990. Larvae were preserved in 95% ethanol. On 12 June, 30 larvae were measured (total length (TL), millimetres) fresh, then preserved and remeasured to estimate size change resulting from preservation. After 1.5 years in ethanol, the relationship between fresh length and preserved length was as follows (units are in millimetres):

$$\text{fresh TL} = -1.556 + 1.037 \times \text{preserved TL}$$
$$(R^2 = 0.992,\ P < 10^{-4})$$

This regression formula was applied to all preserved larvae to estimate fresh lengths.

Additional larvae ($N = 17$) were examined from collections made by the Hudson River Estuary Monitoring Program (HREMP), conducted by a consortium of utility companies. These larvae had been collected in trawls on 3 and 19 July, at km 120–140 (Lawler, Matusky and Skelly Engineers 1992).

Juvenile collections

Juvenile fish were collected by the New York State Department of Environmental Conservation during monitoring surveys from 10 July to 9 November. Collections were made at three beach seine sites (Albany, Poughkeepsie, and Tappan Zee; Fig. 2) with a 30.5-m bag seine, 0.64-cm bar mesh netting, and with channel trawls in the mesohaline portion of the estuary (Haverstraw Bay and Tappan Zee). Trawl collections were made with a 7.9-m headrope Carolina wing bottom trawl (3.8, 3.2, and 1.3 cm in the trawl body, cod end, and cod liner, respectively). Collections were made at 2-week intervals during daylight hours. Where possible, at least 100 fish were taken per sample, although the trawl samples usually yielded ≤20 fish. Juvenile fish were kept on ice until the field crew returned to the laboratory; once in the laboratory, the fish were frozen for later use.

Laboratory methods

Larvae were rinsed in deionized water and keyed out (Leim 1924; Jones et al. 1978; Lippson and Moran 1974) to separate American shad from other clupeids. Shad TL was measured to the nearest 0.1 mm. Larvae were partially cleared with 1% KOH; otoliths were extracted, cleaned in deionized water, and air dried on microscope slides (Brothers 1987). A drop of mineral oil was then placed on the otoliths to secure them.

Total and standard lengths and wet and dry weights (drying overnight at 50–60°C) of thawed juveniles were measured. An index of relative condition was calculated as

$$\text{relative condition} = (W - W')/W'$$

where W is wet weight and W' is expected weight based on length–weight regression of the entire population of juvenile fish ($N = 2102$). Fish that are relatively heavier than the population mean for a given size have a positive condition index, whereas those that are relatively light have a negative index.

Sagittal otoliths of juveniles were extracted, cleaned in 10–20% sodium hypochlorite (bleach), air dried, and embedded in epoxy (Spurr's) with a randomly assigned numerical tag for later referral. The cured plastic stubs were ground to approximately 0.5 mm thickness, and the otoliths were ground to near the midsagittal plane to expose the core. Final polishing was accomplished with a series of increasingly fine lapping papers, down to 3 μm for routine examination.

Otoliths were examined under a compound light microscope with video attachment. For larval otoliths, which are nearly circular, the maximum radius was measured on the video monitor at 290× magnification. For juvenile otoliths, which become asymmetric, three axes (rostral, antirostral, and posterior) were measured at 290×. Increments were counted either at 1080× or under oil immersion (mineral oil, Brothers 1987) at 2660×. Counts were made 2–3 times and averaged. Nearly all counts and increment measurements were made along the major posterior axis, as this area usually has the best resolution (Limburg 1994). Juvenile otoliths were examined "blind," i.e., I did not know the identity of the fish while counting. In all, 280 larval and 1507 juvenile otoliths were read.

Otolith increment widths were measured on most of the

larval and approximately one third of the juvenile otoliths. Increments were marked off on a paper tape directly from the screen; the intervals then were measured with a Grafbar GP-7 sonic digitizer (Science Accessories Corp.) and normalized to the length of the posterior axis.

Hatch dates of shad were estimated by subtracting their estimated ages from the dates on which they were captured. Because fish that hatch closer to the time of capture experience less mortality than older fish, I used Crecco and Savoy's (1987) age-based mortality rates for Connecticut River shad to correct hatch dates for differential mortality. These daily mortality rates (means for 1979–1984) were as follows: 3–9 d, 0.218 d^{-1}; 10–18 d, 0.087 d^{-1}; 19–29 d, 0.056 d^{-1}; and ≥30 d, 0.019 d^{-1}. I assumed that fish <3 d old experienced similar mortality to fish 3–9 d old because Crecco and Savoy (1987) did not provide mortality rates for the former.

Other data

Age distributions from this survey were used to estimate the distributions of different cohorts throughout the Hudson by combining the former with regional standing crops (Lawler, Matusky and Skelly Engineers 1992) computed for the Hudson River Estuary Management Program. Lawler, Matusky and Skelly Engineers (1992) report regional standing crops as estimated by an ichthyoplankton survey, a beach seine survey, and a fall shoals survey, the last two being targeted on juveniles. I chose to use the beach seine survey for this calculation, because juvenile shad generally feed in shore zones during the day (K.E. Limburg, unpublished observations) and because catch efficiencies are higher for beach seines than for the trawl gear used in the fall shoals survey. Only the age distributions of fish collected by beach seine (i.e., not the trawl-collected samples) are included.

Data on lengths of fish collected by the Hudson River Estuary Management Program were obtained from Consolidated Edison of New York, Inc. Records of daily river temperatures at Poughkeepsie, daily freshwater discharges at Green Island, regional river volumes, and regional standing crop estimates for juvenile American shad were taken from Lawler, Matusky and Skelly (1992). Daily water temperature records were obtained from the Niagara Mohawk Power Co. for Albany and from the National Oceanographic and Atmospheric Administration for The Battery, New York City. Weather records for stations in the Hudson River drainage basin were obtained from the Northeast Regional Climatic Center, Cornell University. Zooplankton data were provided by M. Pace, Institute of Ecosystem Studies, Millbrook, N.Y.

Results

Larvae

Larval shad were found at sites from km 89 to km 217. No larvae were found farther downriver, although larvae had been found as far south as km 40 in a preliminary survey conducted in 1989. Larvae were not found at any site on 19 May, and only 5 were collected on 31 May at km 138. Greater densities were found on both 11–12 June and 24 June. Although these collections were not quantitative on an areal or volumetric basis, qualitatively shad larvae were sparser in the shore zone during 1990 than 1989. In both years, shad larvae were found in greatest concentrations in or near sparse aquatic vegetation (*Myriophyllum spicatum* and *Potamogeton crispus*). Larvae usually contained the small cladoceran *Bosmina longirostris* or copepods in their guts.

Table 1. Mean total lengths (TL) and ages of larval American shad collected in the Hudson River estuary, May–June 1990.

Location (river km)	Date	Mean TL (mm)	Estimated age (d)	Est. hatch date	*N*
138	31 May	16.7 (1.5)	25.8 (6.0)	5 May	5
217	11 June	18.4 (2.2)	21.6 (12.6)	20 May	6
138	12 June	19.7 (1.3)	27.3 (2.4)	16 May	42
89	12 June	19.3 (0.7)	27.5 (2.4)	16 May	37
217	24 June	20.7 (0.7)	21.8 (1.0)	2 June	49
177	24 June	19.8 (0.8)	25.1 (2.0)	30 May	59
138	24 June	16.3 (0.3)	20.6 (0.5)	3 June	60
89	24 June	17.4 (2.6)	22.4 (4.6)	2 June	7
137[a]	3 July	18.4 (2.4)	24.7 (4.4)	8 June	16

Note: Values in parentheses are 95% confidence intervals.
[a] Collected by Hudson River Estuary Monitoring Program.

Larval lengths did not vary substantially over time (Table 1); this may have been due to size selection by the sampling gear, although shad ranged in size from 9 mm (within the reported range of hatching sizes, 7–10 mm; Lippson and Moran 1974) to 27 mm. Numerous smaller and larger nonclupeid fish were collected as well. Mean larval ages did not differ or declined somewhat (Table 1). Consequently, estimated hatch dates (Table 1) indicated that successively younger cohorts were collected on each sampling date.

Otolith growth rates, which are positively correlated with somatic growth in American shad (Limburg 1994), are shown for the two sampling dates in June (Fig. 3). Increment widths were small (<3 μm) throughout May, and did not begin to increase until after day 150 (30 May). These low May growth rates corresponded well with the size distributions of shad larvae collected by the Hudson River Estuary Monitoring Program; larval sizes remained stationary throughout the month (Fig. 4). Water temperatures probably played a major role in repressing growth rates. May water temperatures were low (mean at Poughkeepsie = 14.2°C), and a pronounced cool period occurred from day 137 (17 May) through the end of the month.

Juveniles

Recruitment and variable mortality rates

Estimates of hatch dates for all juveniles examined are compared in Fig. 5 with independent estimates (Lawler, Matusky and Skelly Engineers 1992) of life stages that bracket hatching, i.e., eggs and yolk-sac larvae. The relative abundance (expressed as percent frequency) of eggs peaked during the week of 14 May, and yolk-sac larvae during the week of 21 May. Ninety-four percent of all eggs and 81.7% of all yolk-sac larvae were observed in May. However, hatch dates that were back-calculated from juvenile recruits peaked in the period 11–21 June. Mortality-corrected hatch-date estimates did not differ greatly from uncorrected estimates (Fig. 5). When mortality-corrected hatch date estimates were used, only 13.8% of hatches occurred before June.

Fig. 3. Otolith increment widths, averaged over 5-d periods, for American shad larvae at several locations along the Hudson River. Error bars are ±1 SE.

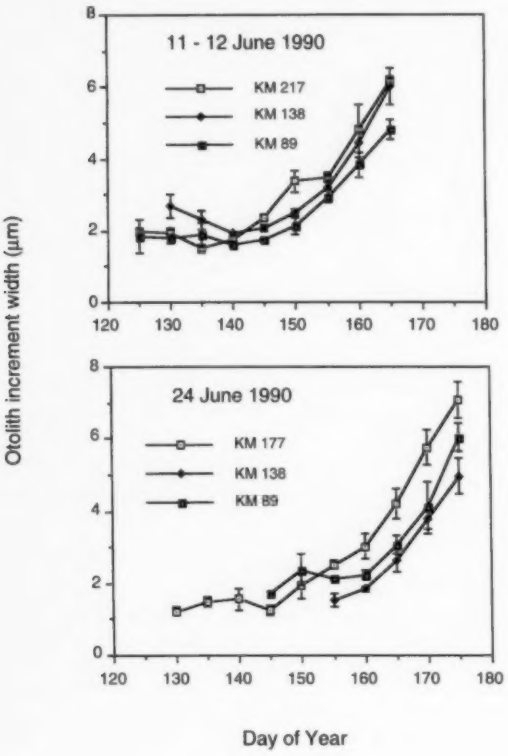


Fig. 4. Total lengths of larval shad versus river kilometre during May 1990. Data source: Hudson River Fisheries Data Base, maintained by Consolidated Edison of New York, Inc.

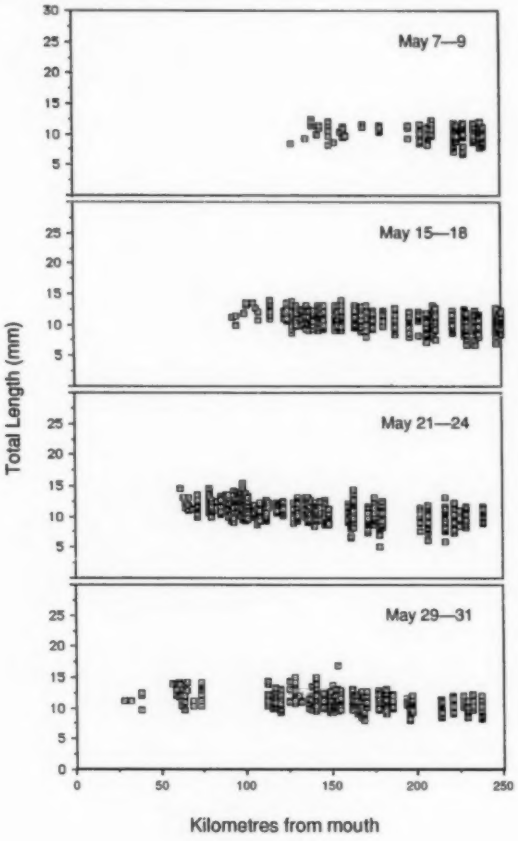


Table 2. One-way analyses of variance for comparisons of hatch dates between Albany (AL) and Poughkeepsie (PK) and Poughkeepsie and Tappan Zee (TZ).

Comparison	Mean hatch date		*F* ratio	*P*	df
Albany vs. Poughkeepsie					
AL 7/10, PK 7/11	160.8 (1.2)	150.5 (1.0)	45.5	$<10^{-4}$	102
AL 7/26, PK 7/24	167.4 (0.6)	159.0 (0.6)	98.0	$<10^{-4}$	186
AL 8/09, PK 8/07	171.6 (0.7)	161.8 (0.6)	119	$<10^{-4}$	142
AL 8/24, PK 8/21	172.7 (0.6)	166.8 (0.8)	37.0	$<10^{-4}$	115
AL 9/10, PK 9/06	170.2 (1.0)	163.2 (1.0)	26.7	$<10^{-4}$	122
AL 9/20, PK 9/18	173.4 (0.9)	163.8 (0.9)	58.2	$<10^{-4}$	117
AL 10/05, PK 10/01	173.3 (0.8)	162.2 (0.9)	86.4	$<10^{-4}$	154
AL 10/18, PK 10/16	175.9 (1.1)	166.0 (1.1)	40.1	$<10^{-4}$	105
Poughkeepsie vs. Tappan Zee					
PK 7/10, TZ 7/18	150.5 (1.0)	148.6 (1.0)	1.99	0.16	126
PK 7/24, TZ 8/02	159.0 (0.8)	159.8 (1.5)	0.21	0.65	107
PK 8/07, TZ 8/14	161.8 (0.7)	158.2 (0.7)	12.6	0.0005	144
PK 9/06, TZ 9/12	163.2 (1.1)	157.7 (1.4)	9.02	0.003	95
PK10/16, TZ10/18	166.0 (1.2)	165.8 (1.3)	0.01	0.914	92
PK 10/30, TZ 10/24	175.9 (3.1)	168.0 (1.4)	5.41	0.023	59

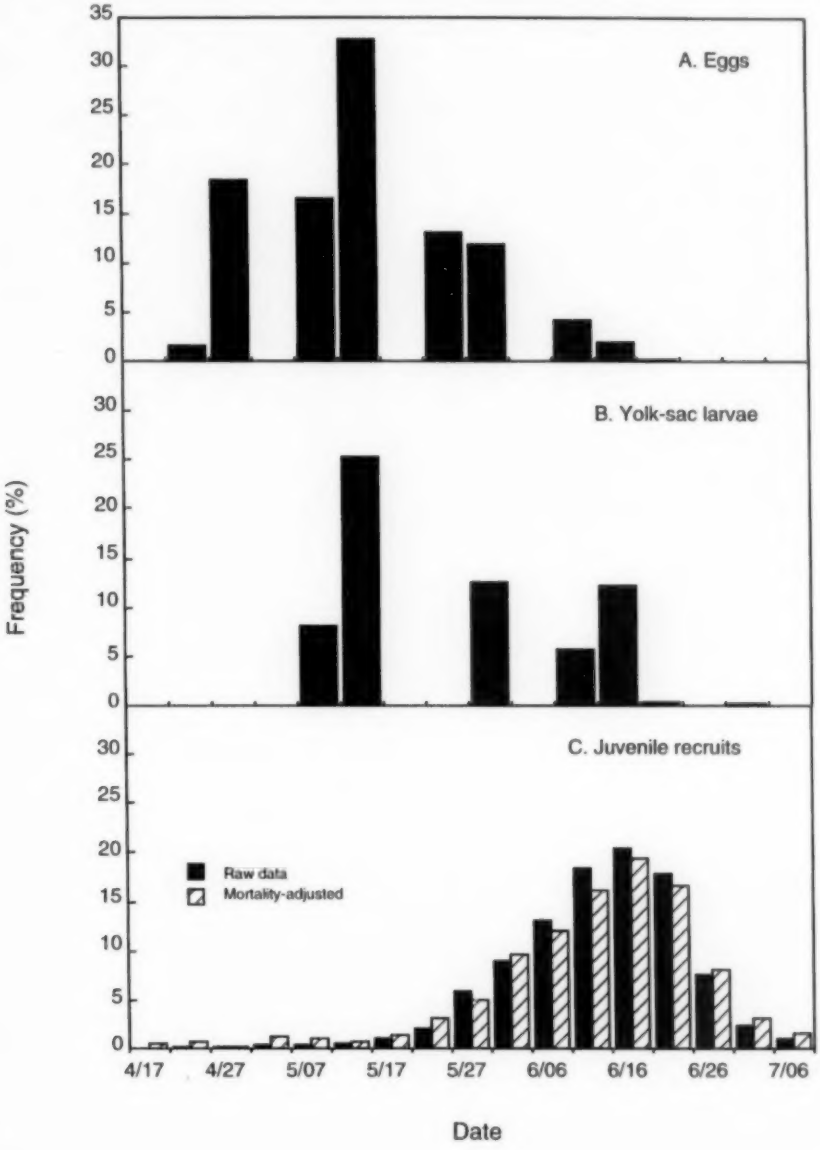
Note: Values in parentheses are the standard error.

Age

At any time prior to mid-October, younger cohorts (indicated by later hatch dates, Fig. 6) predominated upriver and older cohorts were found downriver (Fig. 6). Mean hatch dates at Poughkeepsie were significantly earlier than at Albany for the same collecting period (Table 2). Hatch dates at Poughkeepsie did not differ significantly from hatch dates at Tappan Zee ($\alpha = 0.05$) when comparing July Poughkeepsie samples with Tappan Zee samples the following week, and during mid-October (Table 2). Tappan Zee samples were composed of significantly older cohorts than Poughkeepsie for the periods 7–14 August, 6–12 September, and 24–30 October.

Cohort composition became steadily younger (later hatch dates) at Albany; older (day 160; early to mid-June) cohorts gave way to late June cohorts at later sampling dates (Fig. 6), with some estimated hatches as late as early July. In Poughkeepsie, early June cohorts were replaced by increasingly younger cohorts through August, but from then until mid-October, fish with hatch dates around days 160–165 (mid-June) predominated. Age structure was fairly constant at Tappan Zee until October, when younger cohorts appeared.

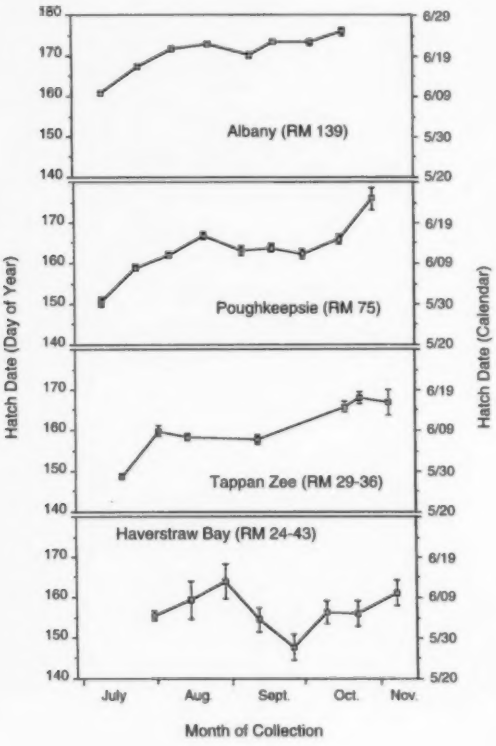
Fig. 5. Temporal frequency (%) distributions of American shad egg and yolk-sac larvae from the entire tidal Hudson River, contrasted with distributions of back-calculated hatch dates of juvenile recruits, 1990: (A) egg relative abundance, (B) relative abundance of yolk-sac larvae, and (C) back-calculated hatch dates of juveniles (both untransformed data and mortality-adjusted estimates; see text for method of calculation) collected in this study. Gaps in egg and larval data are dates not sampled for eggs and larvae. Egg and larval abundance data from Lawler, Matusky and Skelly Engineers (1992).



Sampling was less frequent at Tappan Zee, so the possibility of arrival of younger cohorts in late September cannot be discounted.

Samples collected by trawl in the lower estuary tended to include fewer shad (sample size 17.6 ± 10.8 (mean ± SD)) than beach seine samples. Nevertheless, patterns of hatch dates and age composition could be discerned and were different from the other sites (Fig. 6). Mean hatch dates increased in the first 3 weeks of sampling, declined to a low on 25 September, and rose thereafter. A number of fish collected on 25 September showed anomalies, including early May hatch dates and high growth rates in May (rare in the total population of

Fig. 6. Estimated hatch dates of Hudson River shad, 1990 (means ± SE), versus time of collection. Hatch dates are given both as days of year (left *y* axis) and as calendar dates (right *y* axis).



juveniles). The final sample (8–9 November), containing 40 YOY and 3 yearling shad, had the most heterogeneous age spread of any sample: estimated hatch dates for YOY fish ranged from 17 April to 5 July (Fig. 7).

Total length

Mean TLs are presented in Table 3, and joint distributions of age on length are given in Fig. 7. Mean TL in any given sampling period was smallest in Albany and increased downriver. In cases where length classes overlapped between sites during the same sampling period, older cohorts tended to be located downriver. Conversely, where age-classes had spatiotemporal overlap, larger individuals occurred at downriver sites. At Albany, the first one or two samples appeared to have missed the youngest cohorts because of a gear bias that underrepresented small fish.

Trawl-caught fish in the lower estuary were consistently largest, and showed the greatest heterogeneity in age composition (Fig. 7). By late October, samples in the lower river (Tappan Zee and Haverstraw Bay) included fish >100 mm TL, whereas fish collected upriver never exceeded 98 mm (at Poughkeepsie; Albany maximum, 87 mm). In the final Haverstraw Bay sample (N = 40 YOY plus 3 yearlings), 63% of the YOY exceeded 100 mm TL and 35% were >115 mm TL (up to 157 mm TL, weighing 27.2 g). Ages of these anomalously large (>115 mm) fish ranged from 140 to 205 d, corresponding to estimated hatch dates of day 172 (21 June) and day 107 (17 April), respectively.

Relative condition

Indices of relative condition are given in Table 3, and the joint distributions of relative condition index with age are presented in Fig. 8. Unlike the patterns in length, which showed a more or less monotonic increase over time, relative conditon showed striking upriver–downriver differences. Both at Albany and Poughkeepsie, relative condition rose from initial minima to maxima during the week of 6 August and declined steadily thereafter. At Tappan Zee, relative condition was higher, remaining above zero through mid-August, and declining by mid-October. Relative condition was consistently highest in fishes caught in lower estuary trawls (km 39–69). Despite the similarity in relative condition patterns at Albany and Poughkeepsie, age compositions at Albany and Poughkeepsie were qualitatively different (Figs. 7, 8), as were the size distributions (Fig. 7).

Growth

As reported above, fish lengths tended to be larger downriver at any given time prior to the end of the season. Growth curves for fish hatched in four 5-d cohorts (estimated hatch dates of days 156–160 (4–9 June), 161–165, 166–170, and 171–175) are given in Fig. 9. In each case, growth rates were lowest for Albany and highest for Poughkeepsie and the mesohaline trawl site. However, growth at Albany was similar to that at Poughkeepsie until mid-August (ca. days 220–230). The same pattern can be seen in otolith growth rates (Fig. 10), in which mean 5-d growth rates diverged significantly between Albany and Poughkeepsie fish around day 220 and were dissimilar until mid-September (day 260). Fish caught downriver at Tappan Zee had growth rates intermediate to those at the two upriver sites. Trawl-caught fish from the lower estuary had growth rates similar to those at Poughkeepsie until after day 300 (late October, Fig. 9).

Anomalous growth

On 25 September several fish collected by trawl in the mesohaline zone displayed unusual age and otolith growth patterns. Estimated hatch dates were in late April and early May. These age estimates did not result from mistaking subdaily for daily increments. Thus, these fish potentially represent part of the minority of shad that had survived from that period. However, growth patterns recorded in their otoliths for May did not match the patterns of slow growth observed in otoliths of larvae collected in May and June (Fig. 11). Other survivors from May hatch dates, collected on dates other than 25 September, showed early otolith growth rates similar to those observed in larvae.

Age and standing crop

Table 4 presents estimates of regional standing crops of juvenile American shad from the beach seine surveys of Lawler, Matusky and Skelly Engineers (1992). The increase in standing crop estimates from the first to second weeks was probably due to fish growth, as more fish recruited to the mesh size of the seine; thereafter, total standing crop declined with time.

Table 3. Total lengths (TL) and relative condition indices for juvenile American shad in the Hudson River, 1990.

Week of	AL	PK	TZ	HB
		Total length		
9 July	35.7 (0.8)	42.6 (1.9)	—	—
16 July	—	—	54.4 (0.7)	—
23 July	44.2 (1.0)	49.9 (1.0)	—	—
30 July	—	—	54.4 (1.2)	60.0 (1.9)
6 August	53.4 (0.9)	62.0 (1.0)	—	—
13 August	—	—	61.9 (0.7)	72.3 (8.4)
20 August	56.5 (0.6)	66.3 (1.0)	—	—
27 August	—	—	—	71.1 (3.3)
3 September	—	73.7 (0.6)	—	—
10 September	62.6 (0.6)	—	72.0 (1.2)	77.0 (4.6)
17 September	67.0 (0.6)	77.5 (0.6)	—	—
24 September	—	—	—	80.5 (2.4)
1 October	70.8 (0.9)	81.7 (1.0)	—	—
8 October		—	—	90.2 (5.5)
15 October	73.5 (0.9)	84.5 (0.8)	87.1 (1.4)	—
22 October	—	—	86.6 (1.6)	95.2 (10.6)
29 October	—	79.3 (2.8)	—	—
5 November	—	—	64.7 (2.5)	120.0 (6.3)
		Relative condition		
9 July	–0.193 (0.04)	–0.114 (0.059)	—	—
16 July	—	—	0.032 (0.012)	—
23 July	0.045 (0.016)	–0.057 (0.009)	—	—
30 July	—	—	0.038 (0.032)	0.177 (0.030)
6 August	0.097 (0.018)	0.087 (0.011)	—	—
13 August	—	—	0.066 (0.002)	0.119 (0.042)
20 August	0.043 (0.011)	0.044 (0.023)	—	—
27 August	—	—	—	0.109 (0.061)
3 September	—	0.043 (0.012)	—	—
10 September	0.034 (0.008)	—	—	0.170 (0.041)
17 September	–0.029 (0.005)	–0.003 (0.009)	—	—
24 September	—	—	—	0.047 (0.039)
1 October	–0.026 (0.009)	–0.006 (0.011)	—	—
8 October	—	—	—	0.082 (0.040)
15 October	–0.080 (0.009)	–0.056 (0.009)	–0.098 (0.023)	—
22 October	—	—	–0.043 (0.015)	0.004 (0.059)
29 October	—	–0.084 (0.022)	—	—
5 November	—	—	–0.140 (0.019)	–0.088 (0.009)

Note: Values in parentheses are 95% confidence intervals. AL, Albany; PK, Poughkeepsie; TZ, Tappan Zee; HB, Haverstraw Bay.

Assuming that by 17 July all fish were equally vulnerable to the gear, a regression of total standing crop (thousands of fish) on sampling date (as day of the year) yielded the following regression:

$$\ln(\text{standing crop}) = 11.037 - 0.01402(\text{date})$$
$$(R^2 = 0.92,\ F = 67.0,\ P < 0.001)$$

This regression was used to generate estimates of total standing crop on sampling dates used in the present study. Standing crops of different 5-d cohorts were calculated by using the percentage of total standing crop in a given region (upper (km 151–245, RM 94–152), middle (km 76–150, RM 47–93), and lower (km 19–75, RM 12–46)) for the nearest corresponding date from the Hudson River Estuary Management Program. Age estimates from Albany, Poughkeepsie, and Tappan Zee were used in the upper, middle, and lower reaches, respectively.

From this calculation, numbers of fish in the oldest age-classes were highest in the early samples (Table 5); youngest fish showed peak abundances around the third and fourth sampling dates. There was some indication that fish may have left a region and then returned at a later time. For instance, cohort 156–160 (5–9 June) dominated the upriver standing crop at first (10 July), became diminished and eventually disappeared in late August, and reappeared in small numbers in late September and early October. The numbers of this cohort increased in the mid-Hudson (25 July), though never to the degree originally calculated for 11 July in the upper Hudson;

Fig. 7. Joint distributions of total length and 10-d age cohorts by site and date. Albany (km 241), Poughkeepsie (km 121), and Tappan Zee (km 47–58) are beach seine sites; Haverstraw (km 38–69) is a trawled area of the mesohaline portion of the estuary. Note change in length scale in lower right graphs.

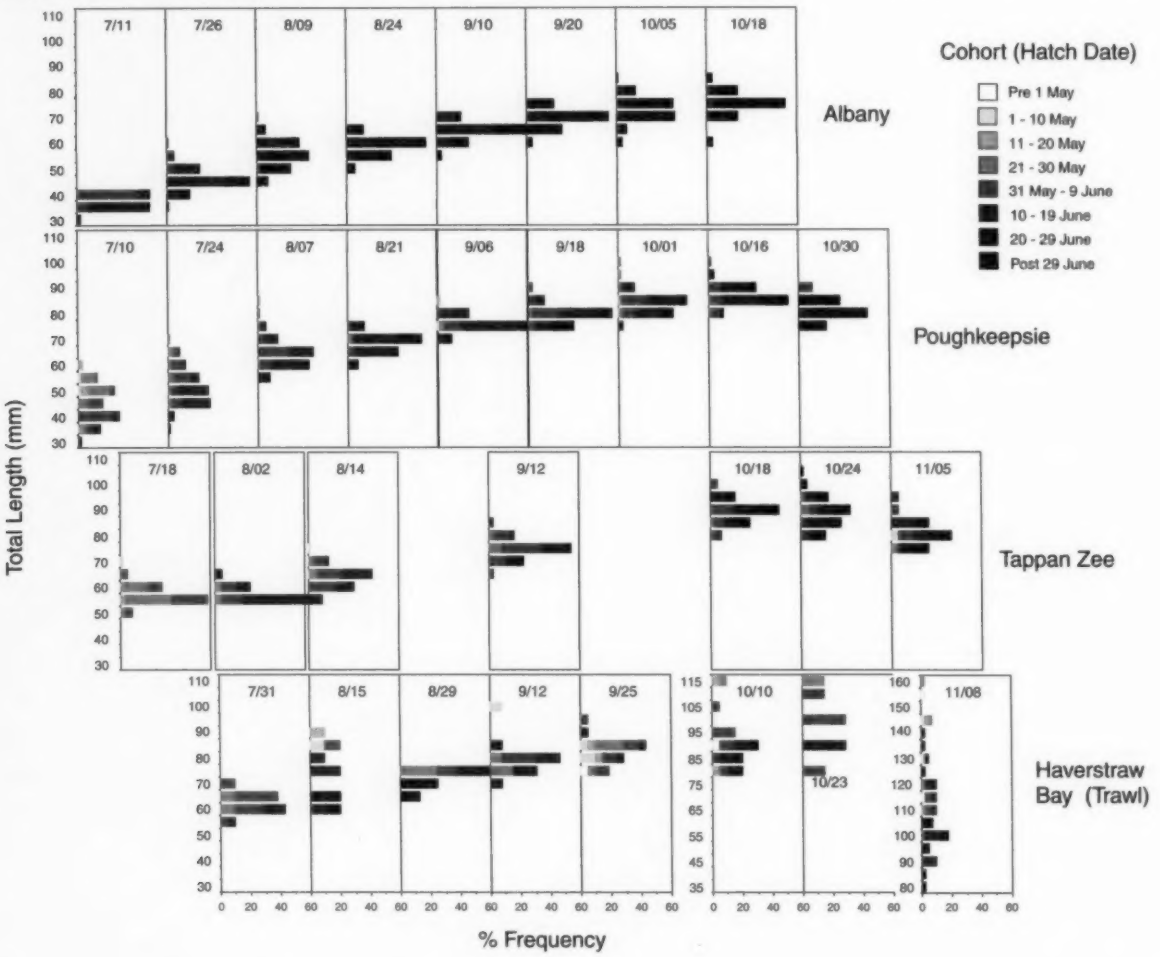


Table 4. Estimated regional standing crops (thousands of fish) of juvenile American shad in the upper (km 154–244), middle (km 76–153), and lower (km 19–75) reaches of the Hudson River, 1990.

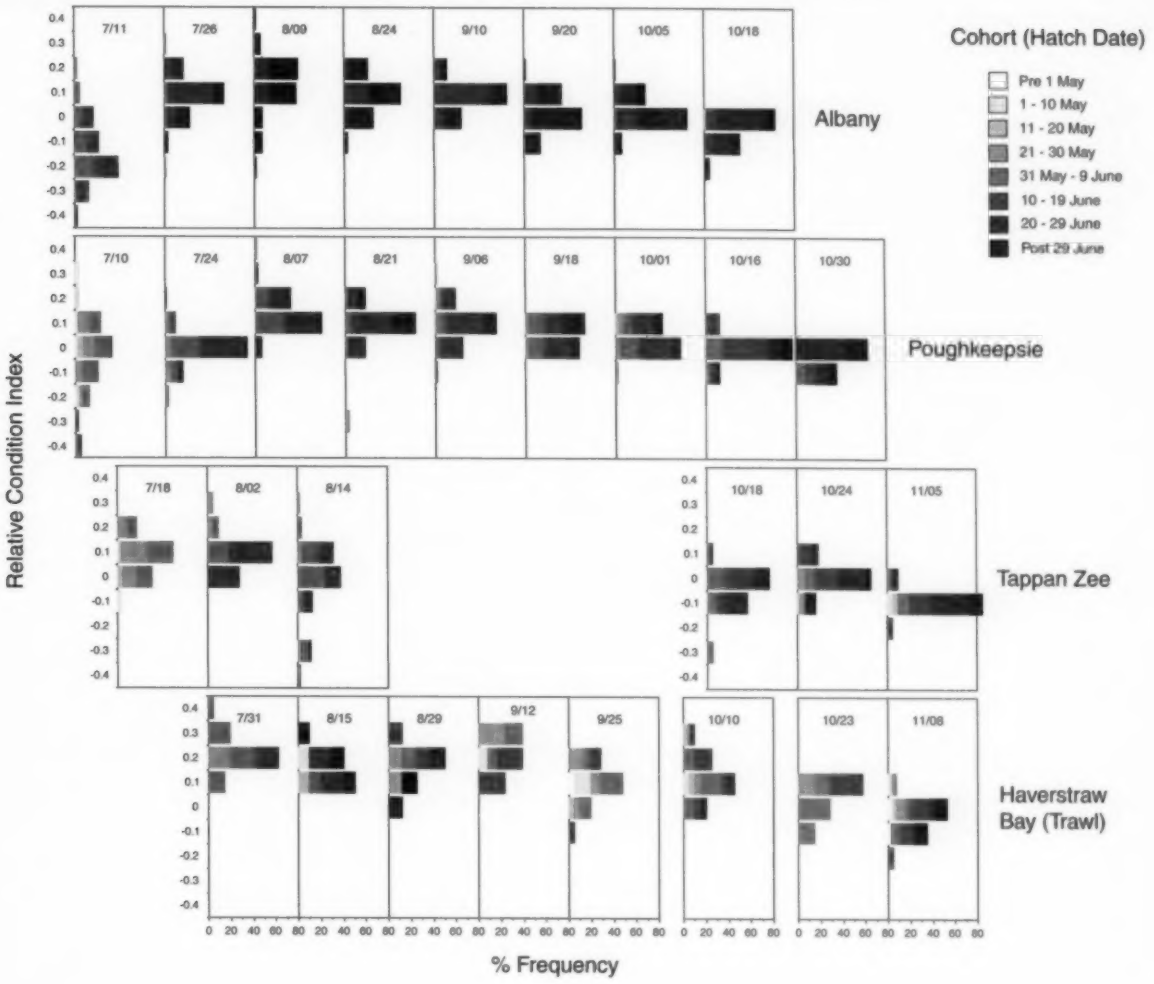
Week of	Upper (%)	Middle (%)	Lower (%)	Total
2 July	1192 (94.6)	68 (5.4)	0 (0.0)	1260
17 July	1370 (30.3)	1758 (38.9)	1392 (30.8)	4520
31 July	2042 (71.3)	521 (18.2)	301 (10.5)	2864
14 August	1448 (64.5)	581 (25.9)	217 (9.7)	2246
27 August	1598 (67.5)	666 (28.1)	104 (4.4)	2368
10 September	880 (58.2)	541 (35.8)	90 (6.0)	1511
24 September	1081 (59.4)	584 (32.1)	156 (8.6)	1821
8 October	831 (68.2)	240 (19.7)	148 (12.1)	1219
22 October	217 (22.8)	193 (20.3)	540 (56.8)	950

Note: Data are from Lawler, Matusky and Skelly Engineers (1992), beach seine surveys only.

similarly, the abundance of this cohort in the lower river generally remained lower than in the upriver regions.

Because of this movement, cohort-specific mortality rates could not be computed. If cohorts were summed over all regions for a given period (e.g., early July, late July, mid-October) and loss rates computed from an exponential decay model, daily loss rates (excluding $\pm\infty$) ranged from 10.8 to –9.9% (Table 6). The negative loss rates may indicate that relative frequencies of age-classes were incorrectly estimated because of undersampling in some weeks, error in age determination, or some unknown source of fish. Calculated cohort abundances cannot be followed explicitly from upriver to downriver sites, with respect to either movement or mortality, with the current sets of assumptions and data. However, if the values within each time interval were averaged, high mean values of 5.5%/d occurred in the first period and declined log linearly to 0.14%/d (Table 6). Time-weighted mean loss rates were highest for cohorts hatched in early May (days 131–135)

Fig. 8. Joint distributions of relative condition and 10-d age cohorts by site and date. See Fig. 7 for details.



and mid-May to early June (days 146–165) and declined for cohorts hatched after day 165.

Tracing migration with an otolith mark

In certain otoliths, a number of the early increments were darker under transmitted light than were later ones. The cause is unknown; however, changes in otolith opacity may correlate with changes in diet and temperature (Brothers 1990). The phenomenon was most common in fish collected in Albany, and hence I called it the "Albany mark." Analysis of the temporal occurrence of Albany marks (i.e., the dates on which the transition from darker to lighter increments occurred) showed an overall mean transition date of 8 July (day 189 ± 6.3, N = 350). The mean transition date occurred later in Albany (10 July) than at the other sites (Poughkeepsie: 5 July, Tappan Zee: 6 July, and mesohaline trawls: 4 July). The spread of transition dates could be due to errors in age determination, to temporally different processes that caused the marks, or, more likely, to a combination of these.

The mark was relatively rare in early samples and increased in frequency over time (Fig. 12*a*). In the Tappan Zee samples, relative frequencies of this mark approached those in Albany in October, as was true of age-classes; both indicate that fishes collected late in the season in Tappan Zee probably were from Albany. Frequencies in Poughkeepsie also rose, but never to the extent observed in Albany. Figure 12*b* shows the fractions of estimated standing crops (calculated as above, and expressed as numbers of fish) possessing the mark. The numbers of fish possessing this mark increased on 9 August in Albany and then declined steadily. If the increase is due to the recruitment of youngest cohorts to the gear, then the gradual decline afterwards may be used to estimate the loss from the Albany area resulting from migration and mortality. From this calculation, the average loss rate from the upper river from 10 August to 19 October is 0.7%/d (range 0.3–1.28%/d).

Table 5. Calculated regional standing crops by 5-d age cohorts (thousands of fish).

Upper (km 154–244)

	Day of year							
Age cohort	10 July	26 July	9 Aug.	24 Aug.	10 Sept.	20 Sept.	5 Oct.	18 Oct.
<120	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
121–25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
126–30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
131–35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
136–40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
141–45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
146–50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
151–55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1
156–60	1966.9	86.1	30.4	0.0	0.0	17.0	17.1	0.0
161–65	1769.4	267.7	182.5	83.5	200.5	33.9	34.3	5.1
166–70	294.2	438.7	484.7	375.9	541.4	118.8	171.7	36.0
171–75	0.0	257.2	969.5	668.3	280.8	424.3	291.9	87.4
176–80	0.0	0.0	332.6	313.3	160.4	288.5	146.0	77.2
181–85	0.0	0.0	30.4	41.8	20.1	33.9	77.3	20.6
>185	0.0	0.0	0.0	0.0	20.1	0.0	17.1	30.9

Middle (km 76–153)

	Day of year								
Age cohort	9 July	25 July	7 Aug.	21 Aug.	6 Sept.	18 Sept.	1 Oct.	16 Oct.	30 Oct.
<120	4.2	0.0	0.0	0.0	8.7	0.0	0.0	0.0	0.0
121–25	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
126–30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
131–35	12.5	15.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
136–40	17.0	0.0	0.0	0.0	8.7	0.0	13.3	0.0	0.0
141–45	12.5	63.6	0.0	13.7	0.0	9.1	13.3	0.0	0.0
146–50	71.1	127.2	20.5	0.0	17.4	27.4	20.0	4.2	0.0
151–55	50.2	190.6	47.7	0.0	17.4	54.8	39.9	16.9	0.0
156–60	58.5	301.9	143.2	54.7	60.7	91.3	73.3	25.4	12.1
161–65	25.1	413.2	150.1	164.1	182.3	127.9	119.8	59.2	0.0
166–70	4.2	222.4	129.6	218.8	156.2	118.7	93.2	55.0	12.1
171–75	0.0	47.7	27.3	136.7	78.1	118.7	46.6	29.6	36.3
176–80	0.0	0.0	0.0	27.3	0.0	27.4	13.3	8.5	36.3
181–85	0.0	0.0	0.0	0.0	0.0	0.0	6.7	16.9	12.1
>185	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.2

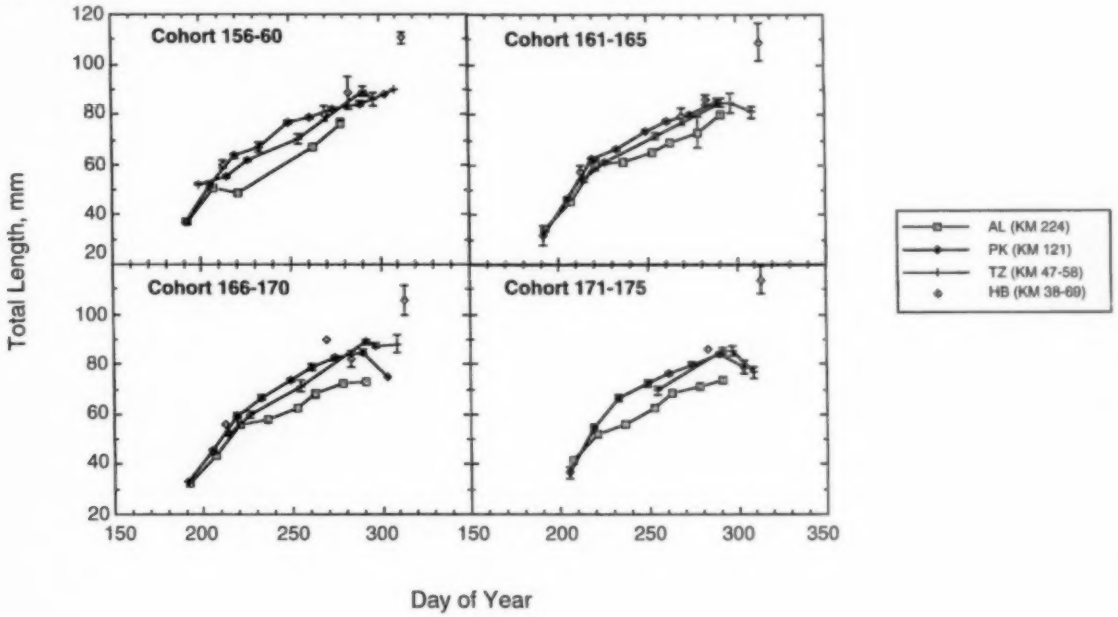
Lower (km 19–75)

	Day of year						
Age cohort	18 July	2 Aug.	14 Aug.	12 Sept.	18 Oct.	24 Oct.	5 Nov.
<120	0.0	0.0	0.0	0.0	0.0	0.0	0.0
121–25	0.0	0.0	0.0	0.0	0.0	0.0	38.7
126–30	18.2	0.0	0.0	0.0	0.0	0.0	0.0
131–35	54.6	0.0	0.0	0.0	0.0	0.0	0.0
136–40	18.2	0.0	7.6	0.0	0.0	0.0	0.0
141–45	181.8	0.0	0.0	3.0	10.0	22.4	38.7
146–50	400.0	32.3	15.1	8.9	0.0	22.4	0.0
151–55	400.0	48.6	56.8	38.7	49.9	33.7	77.4
156–60	109.1	32.3	87.0	17.9	80.0	56.1	38.7
161–65	0.0	178.0	60.5	20.9	60.0	44.9	116.2
166–70	0.0	48.6	34.0	8.9	89.9	123.5	77.4
171–75	0.0	0.0	0.0	6.0	70.0	123.5	116.2
176–80	0.0	0.0	0.0	0.0	49.9	67.3	193.6
181–85	0.0	0.0	0.0	0.0	10.0	56.1	38.7
>185	0.0	0.0	0.0	0.0	0.0	0.0	38.7

Table 5 (*concluded*).

Total						
	Day of year					
Age Cohort	14 July	29 July	10 Aug.	9 Sept.	17 Oct.	4 Nov.
<120	4.2	0.0	0.0	8.7	0.0	0.0
121–25	4.2	0.0	0.0	0.0	0.0	40.2
126–30	18.2	0.0	0.0	0.0	0.0	0.0
131–35	67.1	15.9	0.0	0.0	0.0	0.0
136–40	35.2	0.0	7.6	8.7	0.0	0.0
141–45	194.3	63.6	0.0	3.0	10.0	40.2
146–50	471.1	159.5	35.6	26.3	4.2	0.0
151–55	450.2	239.2	104.5	56.1	72.0	80.4
156–60	2134.5	420.3	260.7	78.6	105.3	40.2
161–65	1794.5	858.8	393.2	403.7	124.4	120.6
166–70	298.4	709.7	648.4	706.6	181.0	80.4
171–75	0.0	304.8	996.8	364.8	187.0	120.6
176–80	0.0	0.0	332.6	160.4	135.6	201.0
181–85	0.0	0.0	30.4	20.1	47.5	40.2
>185	0.0	0.0	0.0	20.1	30.9	40.2

Fig. 9. Mean total lengths of fish in similar age cohorts (referenced by hatch dates) at different sampling sites, 1990: cohort 155–160 (4–9 June), cohort 161–165, cohort 166–170, and cohort 171–175. AL, Albany; PK, Poughkeepsie; TZ, Tappan Zee; HB, Haverstraw Bay.



Discussion

Larval and juvenile distributions and movements

Major differences in hatch-date representation were observed between the larval and juvenile surveys. Larvae were mostly caught in June and had estimated hatch dates ranging from early May to early June, while over half the recruited juveniles were estimated to have hatched after mid-June, and May-hatched juveniles were rare. Larvae that spawned later may have been small enough to have been extruded through the seine mesh, although this seems unlikely as other small larvae (*Morone* spp., typically 5–7 mm) were retained.

Alternatively, yolk-sac larvae may not use the inshore habitat as extensively as post yolk-sac larvae. American shad eggs are semibuoyant to demersal and drift along river bottoms (Marcy 1976). Mitchell (1925, cited in Crecco et al. 1983)

Fig. 10. Otolith growth rates for age cohort 166–170 (15–19 June) for Albany (AL) and Poughkeepsie (PK). Growth rates are expressed as mean 5-d otolith increment widths (±2 SE) for fishes within this age cohort.

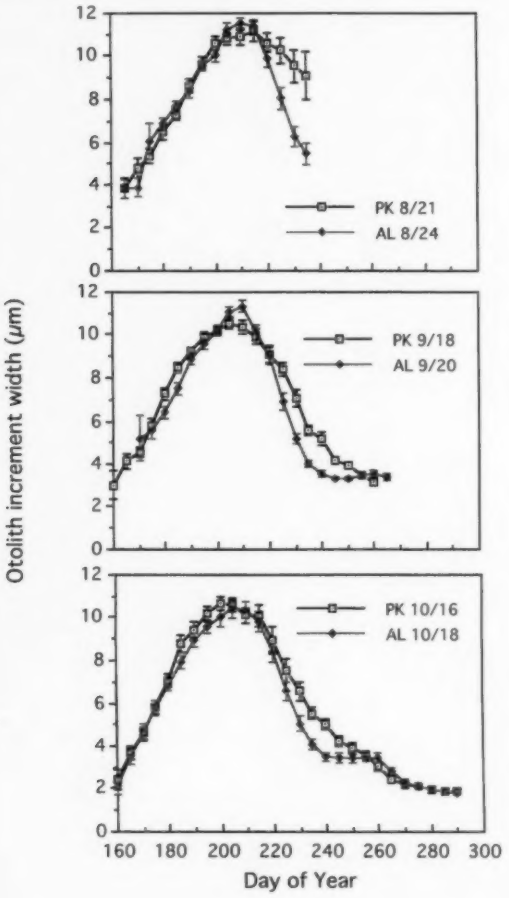
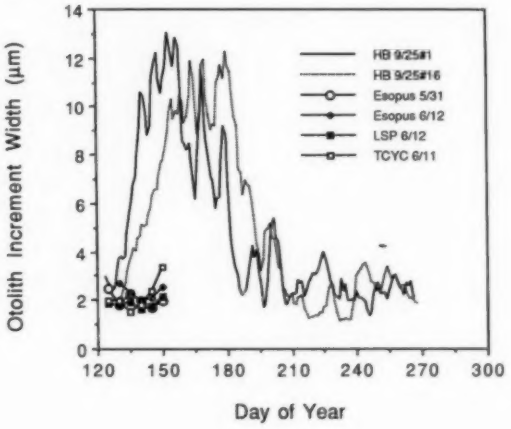


Fig. 11. Patterns of growth in otoliths from two juvenile shad collected in Haverstraw Bay, 25 September 1990, compared with otolith growth rates of larvae collected in May and June 1990. Each larval growth curve represents means of at least 30 larvae.



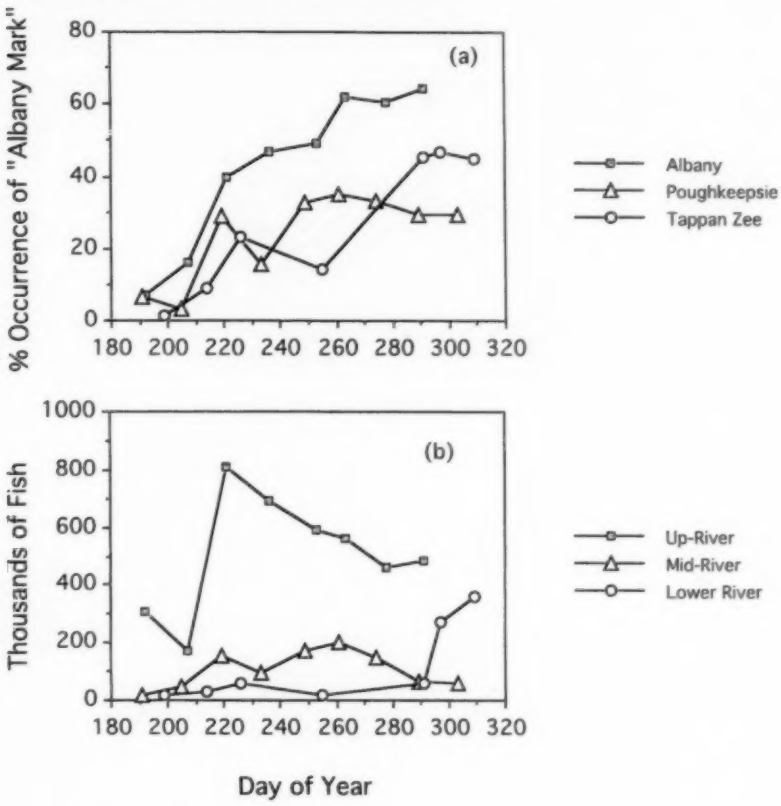
reported that yolk-sac larvae remain near the bottom and swim to shore as the yolk-sac resorbs. Metzger et al. (1992), in a study of habitats not usually sampled in the Hudson River Estuary Management Program, found more yolk-sac larvae offshore in shoals (3–6 m) and bottom habitat than inshore, and the reverse for post yolk-sac larvae. Larvae with May hatch dates were found at the furthest upriver site (km 217, RM 135; Table 1), but these early cohorts were never found upriver in the juvenile survey.

Movement of juvenile shad downstream appears to have strong age and size components, supporting the first and second hypotheses stated in the Introduction. Actual migration rates are difficult to compute reliably. However, general patterns can be identified, e.g., the times that certain cohorts appear in downriver samples compared with upriver ones. Generally, the appearance in Albany of cohorts hatched successively later was followed in Poughkeepsie 2 weeks later. This was also true of cohort appearances at the downriver sites, although sampling occurred 1 week later there. At the same time, cohorts that had hatched earlier gradually disappeared from up- and mid-river samples and were replaced by cohorts that had hatched later. This pattern suggests that cohorts do move downriver as a function of age. Moreover, within an age cohort, larger individuals were observed downriver (Fig. 7) during most sampling periods, at least until mid-October: size-at-age (a reflection of growth rate) is thus a second factor in downstream movement. In the final samplings, the reduction in mean size at Tappan Zee and Poughkeepsie, coupled with age distribution data and the fact that fish were not present in Albany, is evidence that the last fish leaving the system consisted of the youngest cohorts from upriver. Rapidly falling water temperature is likely to "push" the last cohorts out of the river in late October–November (Chittenden 1969; Limburg 1994).

The size and age distributions of fish caught by trawl in the mesohaline zone are more difficult to interpret. Although fewer in number, these fish were consistently larger and represented a broader mix of age-classes. On 25 September a number of fish, while not significantly larger than others in the sample, showed unusually high otolith growth rates in May. As the entire Hudson River was subject to cool temperatures during May, which was reflected in low otolith growth rates of larvae and other shad juvenile recruits, high May growth rates may indicate that these fish had located a thermal refuge somewhere in the Hudson during May. Alternatively, these fish may have been spawned in a different, warmer natal river, and moved into the Hudson after a sojourn at sea. This latter explanation is supported by a study of otolith microchemistry, reported elsewhere (Limburg 1995).

How do cohorts move downstream? Certainly, larvae can be advected by high river discharge, as seen in Fig. 4. However, if all fish moved passively, using downstream advection, rates of cohort appearance at downstream sites should have mirrored freshwater transit times. As seen in Table 7, summertime water transit times were slow (July 1990 flows were

Fig. 12. Occurrence of Albany mark, a mark seen on early increments of otoliths. (*a*) Percent occurrence in samples; (*b*) numbers of fish with Albany mark per river region, using standing crop estimates calculated as described in text.



comparable to those in June); it would take on the order of 12–16 weeks for a passively drifting fish to travel from Albany to Tappan Zee. However, cohorts appear 2–3 weeks later downstream than upstream, suggesting an active migration during the summer months. This observed downstream movement occurred well before temperatures declined to 19°C, which does not support the hypothesis that downstream migration is cued solely on declining fall temperatures (O'Leary and Kynard 1986).

The serendipidous presence of a mark on otoliths of fish originating upriver at Albany (the Albany mark) provided an estimate of loss of fish from that river section (0.7%/d). Declines in the numbers of fish with this mark from the upper Hudson were tracked by corresponding increases in marked fish in the central Hudson, which suggests that migrants from upriver were seen proportionately in the mid-Hudson collections. However, these fish were not seen in equal proportion in the lower Hudson (Fig. 12*b*). The discrepancy may be due to differential mortality between the middle and lower river reaches. Alternatively, migrants from the mid-Hudson may tend not to use nearshore habitats en route to the sea and thus would be underestimated by beach seine samples taken in the lower river.

Relative condition and growth

Relative condition indices showed a similar pattern in the Albany and Poughkeepsie samples: low values at first, peaking in early August, and declining into the fall. This pattern was not coupled to size or age, but rather showed a strong temporal correlation. Curiously, relative condition indices remained high during August (Fig. 8), while both somatic (TL) and otolith growth rates declined in Albany fish relative to Poughkeepsie fish at that time (Figs. 10, 11). Albany fish were therefore relatively heavier than Poughkeepsie fish for that period.

The relative condition of downriver fish was initially higher than for upriver and midriver fish; highest condition index values were observed in fish trawled in the mesohaline zone, remaining above zero until November (Table 3). The lower reach of the tidal Hudson (mesohaline zone) is more productive (as evidenced by primary productivity and respiration rates) than the upper and middle reaches (Weinstein 1977; Sirois and Fredrick 1978; Howarth et al. 1992). The greater production of the lower estuary appears to be translated into higher fish production (Limburg 1994) and may also be reflected in the higher condition indexes of juvenile shad caught in this reach.

Table 6. Calculated whole river loss rates (%/d) for 5-d age cohorts (indexed by hatch dates) of juvenile American shad.

5-d cohort (by hatch date)	Intervals (days of year)					Time-weighted cohort mean
	July 13–27	July 27–Aug. 9	Aug. 9–Sept. 8	Sept. 8–Oct. 17	Oct. 17–Nov. 4	
131–135	9.6	—	—	—	—	9.6
136–140	—	—	–0.5	—	—	–0.5
141–145	7.4	—	—	–3.2	–7.7	–2.3
146–150	7.2	12.5	1.0	4.8	—	5.0
151–155	4.2	6.9	2.1	–0.7	–0.6	1.5
156–160	10.8	4.0	4.0	–0.8	5.3	3.4
161–165	4.9	6.5	–0.09	3.1	0.2	2.4
166–170	–5.8	0.8	–0.3	3.6	4.5	1.2
171–175	—	–9.9	3.3	1.8	2.4	0.8
176–180	—	—	2.4	0.4	–2.2	0.6
181–185	—	—	1.4	–2.3	0.9	–0.4
>185	—	—	—	–1.1	–1.5	–1.2
Mean ± SE	5.47 ± 2.08	3.47 ± 3.10	1.48 ± 0.54	0.56 ± 0.84	0.14 ±1.30	1.67

Note: Dates represent means for the dates used in individual regional calculations. Loss rate is computed as: $Z = -\ln(N_{t_{current}} / N_{t_{previous}}) / (t_{current} - t_{previous})$ where N is standing crop (in thousands) and t is time (day of year). Negative numbers are negative loss rates.

Table 7. Calculated discharge transit times through the Hudson River, March–June, 1990.

Week of	Weekly Green Island discharge (10^6 m^3/week)	Mean regional transit times (weeks)			
		Upper	Middle	Lower	Total
1 March	310.176	1.3	3.1	2.9	7.3
8 March	498.355	0.8	1.9	1.8	4.6
15 March	1 074.816	0.4	0.9	0.8	2.1
22 March	617.846	0.7	1.5	1.5	3.7
29 March	501.120	0.8	1.9	1.8	4.5
5 April	691.200	0.6	1.4	1.3	3.3
12 April	639.792	0.6	1.5	1.4	3.5
19 April	396.058	1.0	2.4	2.3	5.7
26 April	322.099	1.3	3.0	2.8	7.0
3 May	283.824	1.4	3.4	3.2	8.0
10 May	600.048	0.7	1.6	1.5	3.8
17 May	1 066.176	0.4	0.9	0.9	2.1
24 May	568.598	0.7	1.7	1.6	4.0
31 May	289.526	1.4	3.3	3.1	7.8
7 June	143.424	2.8	6.6	6.3	15.8
14 June	264.038	1.5	3.6	3.4	8.6
21 June	120.269	3.4	7.9	7.5	18.9

Note: Upper reach spans km 150–245 (RM 93–152), middle reach spans km 76–149 (RM 47–92), and lower reach spans km 19–75 (RM 12-46). The regional river volumes were as follows: upper, 408 176 559 m^3; middle, 952 334 777 m^3; lower, 907 304 773 m^3; total, 2 267 816 109 m^3.

Relative condition declined in the fall at all sites. Although lipid content was not measured, the amount of fat stored in the body cavity of fish was observed to diminish as well. Also, the occurrence of acanthocephalan parasites was observed more frequently in the intestines of late-season fish (K.E. Limburg, unpublished data), and caloric values of shad juveniles declined into the fall (Limburg 1994). These observations raise the question of what adaptive advantages accrue to juvenile shad by remaining in the river as environmental conditions worsen.

Table 8. Development times of American shad eggs at different water temperatures.

Temperature (°C)	Development time (d)	Source
12	13.5	Leim 1924
12	17	Ryder 1887[a]
16.1	7	Hendricks[b]
17	7	Leim 1924
27	2	Rice 1878[a]

[a]Cited in Boreman (1981).

[b]M. Hendricks, Van Dyke Fish Hatchery, Pennsylvania Fish Commission, personal communication.

Differential recruitment of cohorts

In this study, the age structure of shad that recruited successfully to the juvenile stage differed considerably from the temporal distributions of eggs and yolk-sac larvae (Fig. 5). Possible reasons for the discrepancy include temperature-dependent lags in egg development time, underestimation of the true ages of recruited juveniles, and differential recruitment of cohorts.

Hatching of American shad eggs is successful between temperatures of about 11–12 and 27°C (Leim 1924). Within that range, egg development time is a monotonic decreasing function of temperature (Table 8) and can be expressed as

$$\ln(\mathrm{EDT}) = 8.9 - 2.484 \times \ln(T)$$

where EDT is egg development time in days and T is temperature in degrees Celcius ($R^2 = 0.987$, $F_{[1,4]} = 223$, $P < 0.0001$). At 12°C, predicted egg development is 15.5 d with a 95% confidence interval range of 12.4–19 d; at 16°C, it is 7.6 d (6.5–8.5 d); and at 20°C, it is only 4.3 d (3.5–5.1 d).

Hudson River temperatures in May varied between 12.5°C (1 May) and 15.9°C (14 May). The observed lag between egg presence and back-calculated hatch dates (Figs. 5*a*, 5*c*) is not well explained by egg development times, because the lag is much larger (35 d between the mode of egg abundance and

Fig. 13. Daily freshwater discharge at Green Island, N.Y., and Hudson River water temperature at Poughkeepsie, N.Y., May and June 1990. Data source: Lawler, Matusky and Skelly Engineers (1992).

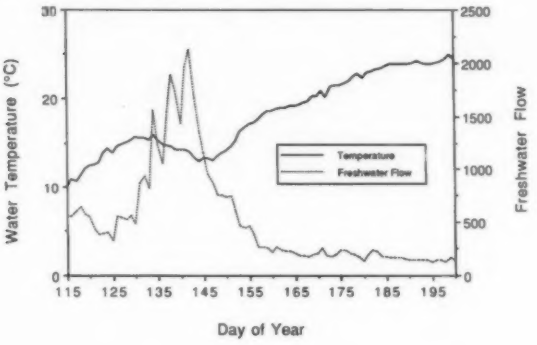
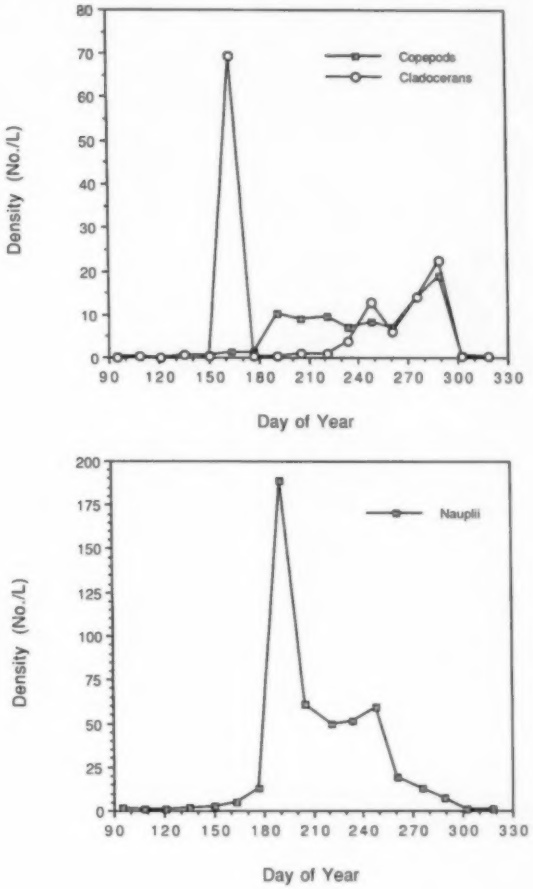


Fig. 14. Densities (no./L) of Hudson River zooplankton collected near Kingston, N.Y. (RM 90, km 146), 1990. Data source: M.L. Pace, Institute of Ecosystem Studies, Millbrook, N.Y.



that of back-calculated hatch dates) than predicted by the above regression equation. The discrepancy between the temporal distribution of recently hatched yolk-sac larvae and estimated hatch dates of recruited juveniles (Figs. 5*b*, 5*c*) is even stronger evidence of poor survival of early cohorts and better survival of later ones.

Underestimation of the true ages of juvenile recruits could also potentially cause such a discrepancy. Age underestimation appears to be more common than overestimation (Beamish and McFarlane 1983; but note that overestimation can potentially occur if subdaily increments are mistaken for daily ones: care was taken not to do so in this study). In a validation study of age determination with juvenile shad otoliths (Limburg 1994), some otoliths exhibited a blurred zone that corresponded to the period of adjustment from pond to indoor laboratory conditions; increments were difficult to count in this zone and resulted in underestimation of age by 3–5%. On the other hand, otoliths from pond-reared fish were clear and resulted in counts that were accurate to within 1 d (up to 190 d). Although some age underestimation in the present study is likely, it probably is less than 10%. Even if a long (20 d) egg development time is assumed, a 10% underestimation in age cannot explain the discrepancy between temporal distributions of eggs and back-calculated hatch dates of recruited juveniles.

Errors notwithstanding, it appears that cohorts that spawned later recruited more successfully into the juvenile stage than did cohorts that spawned earlier. This may be the result of a combination of abiotic and biotic factors. Freshwater discharges in May 1990 from the upper Hudson (the primary input) rose dramatically in midmonth, with a consequent drop in water temperatures (Fig. 13). Weather records for 1990 from New York State weather stations in the Hudson drainage (Albany, Utica, Glens Falls, Plattsburgh, and Poughkeepsie) indicate drainage-wide heavy rainfalls on 13, 16, 17, 20, and 21 May. With data on volumes of different reaches of the Hudson (Lawler, Matusky and Skelly Engineers 1992), calculations of mean transit times of discharge (Table 7) show very rapid water turnover during this time. Even though shad larvae presumably possess behavioral mechanisms to avoid being flushed out of nursery areas (otherwise on average all shad would be gone from the Hudson by August), these high May flows appear to have advected many eggs and larvae downstream (Fig. 4).

Conditions in June were considerably more benign, with warmer temperatures, lower flow rates, and high zooplankton densities (Fig. 14). Zooplankton, primarily copepods and *Bosmina* sp., are the primary food of larval American shad (Crecco and Blake 1983; K.E. Limburg, unpublished data). The peaks in abundance of cladocerans (mainly *Bosmina longirostris*) and copepod nauplii (Fig. 14) correspond well to times of the larval stages of recruited juveniles. Early otolith increments were much wider (up to 10×) for fish hatched after mid-June compared with May cohorts, reflecting the more amicable environment.

Thus, it is postulated that high May river discharges, and associated low temperatures and low food availability, contributed to high larval mortality, and that larvae hatched after May experienced favorable hydrodynamic, thermal, and feeding conditions. Post yolk-sac larvae concentrate in quiet areas

near shorelines (Cave 1978; Metzger et al. 1992). The high May river flows may have swept larvae out of quiet backwaters and exposed them to turbulent conditions, to which American shad larvae may be sensitive (Limburg 1994). Also, low growth rates of larvae in May are likely to have increased their exposure time to predators as has been suggested by Rice et al. (1987) for bloater (*Coregonus hoyi*) larvae.

The causes of larval fish mortality and variable recruitment are subjects of critical importance in fisheries biology that have been reviewed extensively (cf. Hjort 1914; May 1974; Cushing 1981; Lasker 1981; Rothschild 1986; Anderson 1988; Houde 1989). In marine ecosystems especially, larval survival has been linked to food availability (the critical period hypothesis: Hjort 1914; May 1974), to advection to favorable or unfavorable areas (Cushing 1975, 1990; Houde 1989), and to size-related phenomena, principally predation (Peterson and Wroblewski 1984; McGurk 1986; Miller et al. 1988; Pepin 1988–1989; Pepin and Myers 1991). In the Connecticut River, Crecco and colleagues demonstrated that (*i*) mortality rates of subyearling American shad diminish rapidly after the larval phase (larvae, 19–26%/d; juveniles, 1–2%/d; Crecco et al. 1983, 1986; Crecco and Savoy 1987), (*ii*) larval survival is affected by hydrographic events (Crecco and Savoy 1985) and is inversely correlated with June river flows and positively correlated with June river temperatures (Savoy and Crecco 1988), and (*iii*) survival varies in proportion to May–July zooplankton densities (Crecco et al. 1983). This study, although consisting only of data from a single year, corroborates the findings of the Connecticut River studies.

Despite the apparent high loss rates of shad eggs and larvae in the Hudson River, the 1990 recruitment does not appear to be an unusually bad one. In three indices of juvenile year-class strength compiled from 1979 to 1991, 1990 ranked third (New York State Department of Environmental Conservation American shad monitoring program; New York State Department of Environmental Conservation 1992), fourth (Hudson River Estuary Monitoring Program beach seine survey; Lawler, Matusky and Skelly Engineers 1992), and sixth (Hudson River Estuary Monitoring Program fall shoals survey; Lawler, Matusky and Skelly Engineers 1992), respectively.

In this close look at the 1990 year-class of juvenile American shad, evidence of directed downstream movements was seen as early as June–July. Older cohorts gradually moved downstream during the summer and into the fall. In late fall, all cohorts were observed to move down. Within age cohorts, larger individuals appeared to move downriver ahead of smaller ones. The year-class was established by cohorts hatched after 1 June, although highest abundances of larvae were recorded by monitoring programs in May. Finally, juvenile shad of different sizes, ages, and possibly origins use the lower estuary channel in the fall. Further research into juvenile outmigration should include the fate of outmigrants as a function of migration time (early versus late) and between-river comparisons of migration patterns.

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Instructions to Authors

Canadian Journal of Fisheries and Aquatic Sciences

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together.

The *Journal* considers the following types of contributions:

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Perspectives — Essays of opinion or hypothesis on aquatic science of concern to the professional and layperson. References are desirable; informal style is welcome.

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Manuscript submission

Submit manuscripts in triplicate to the Editor, *Canadian Journal of Fisheries and Aquatic Sciences*, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada, accompanied by a covering letter that (1) states the main points and significance of the work, (2) avows that all coauthors fully participated in and accept responsibility for the work and that it is not being considered for publication elsewhere, (3) suggests potential referees, (4) identifies other manuscripts containing the same or similar information, and (5) includes the telephone and facsimile numbers and e-mail address of the corresponding author. Original typescripts and figures (except halftones) are best retained by the author until requested. **Packaging of manuscripts and illustrations should be robust enough to resist damage in transit.**

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal*'s editorial policy or scope.

Authors are requested to submit diskettes (with *accepted* manuscripts only) prepared on Macintosh or IBM-compatible personal computers. Include files in both a word-processing format (WordPerfect or Word is preferable) and ASCII on a 3.5- or 5.25-in. disk. Please identify the word-processing software and version number used.

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The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103, U.S.A.). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed. 1991. Spec. Publ. No. 20, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990. Trans. Am. Fish. Soc. 119: 2–15). All measurements must be metric, use SI symbols in most instances (exceptions noted in the editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1–79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, ON M9W 1R3, Canada).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. **Alterations to the content must be avoided.**

Preparation of manuscripts

General guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. General guidelines follow.

Type the manuscript on white paper (8.5 × 11 in.) on only one side of the page. Leave all margins at least 1 in. wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including

tables, captions for tables and figures, footnotes, and the reference list. Use italic font if available; when not available, underline material that is to be set in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what readers need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

(1) Limit abstract to one paragraph of about 175 words.

(2) Double-space all elements of the manuscript, including references, table captions, and figure legends.

(3) Avoid exclusive use of capitals anywhere in the manuscript, including headings, table captions, and figure legends.

(4) Italicize or underline only Latin names of organisms and appropriate statistical and mathematical notations.

(5) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

(6) Avoid using ambiguous forms such as g $C/m^2/d$; use g $C \cdot m^{-2} \cdot d^{-1}$.

(7) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

(8) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

(9) Include acknowledgements section at manuscript stage, not when page proofs arrive.

(10) Provide the person's initials and mailing address when referring to personal communications.

(11) Delete unnecessary references that do not apply directly to the problem.

(12) Check references carefully against text citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g., available from Department of Economics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

(13) Delete commas between name and date in citations such as (Smith 1990) and do not underline "et al."

(14) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g., thousands).

(15) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints or laser printouts of figures, or originals if they do not exceed 8.5 × 11 in., on request for publication. Use the same type of lettering throughout if possible.

Parts of the manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g., X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . . " or "Investigations on . . . " Good titles greatly assist scientists and librarians in using scientific literature and aid indexers in preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the contents and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those

needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as " . . . is discussed" or " . . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents and be completely self-contained (i.e., any *essential* references) in a retrievable form (e.g., R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. 37: 268–282).

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and methods

Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . . " If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

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References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence**. Nearly every manuscript submitted to the *Journal* contains errors in the references.

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Tables are used to present repetitive data and should be as economical of space as possible.

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Indicate table footnotes by superscript lowercase letters and type them below the table.

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Provide photographic reproductions, laser printouts, or the original artwork (no larger than 8.5 × 11 in.) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

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Leave three spaces above and below lines that have much superscript material, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between lowercase *l* and the numeral *one*, and between capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	(Bq)
calorie	(cal)
centimetre	(cm)
centimetre, square	(cm^2)
centimetre, cubic	(cm^3)
centimetres per gram per second	($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb	(C)
day	(d)
decimetre	(dm)
degree Celcius	(°C)
degrees of freedom	(df)
gram	(g)
hectare	(ha)
hertz	(Hz)
hour	(h)
joule	(J)
kilometre	(km)
litre	(L)
lumen	(lm)
lux	(lx)
metre	(m)
metre, square	(m^2)
metre, cubic	(m^3)
micrometre	(µm)
milligram	(mg)
millilitre	(mL)
millimetre	(mm)
millimetre, square	(mm^2)
minute	(min)
molar mass	(M)
mole	(mol)
moles per litre	(mol/L, M)
month	(mo)
Pascal	(Pa)
second	(s)
standard deviation	(SD)
standard error	(SE)
tonne (metric ton)	(t)
volt	(V)
volume	(vol)
watt	(W)
week	(wk)
year	(yr)

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be used with the year offset by commas (On October 9, 1983, the . . .).

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Reference lists

References should be listed at the end of the paper in alphabetical order according to surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. **39**: 1444–1452.

Entire issue of journal

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Book in a series
Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Book not in a series
LeBlond, P.H., and Mysak, L.A. 1978. Waves in the ocean. Elsevier, New York.

Part of book
Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. Neil and D.C. Himsworth. Oregon State Unversity Press, Corvallis, Oreg. pp. 203–229.

Corporate author
American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C.

Theses
Kutty, M.N. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports
Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can.Tech. Rep. Fish. Aquat. Sci. No. 1032.

Translation
Koike, A., and Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. (Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word list

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Recommendations aux auteurs

Journal canadien des sciences halieutiques et aquatiques

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sousjacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles — études d'une portée générale, constituant des contributions inédites à la science.

Critiques — appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives — essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Commentaires — opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur, *Journal canadien des sciences halieutiques et aquatiques*, Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada, **en triplicata** et accompagnés d'une lettre qui fait état (1) des principaux points et de l'importance du travail, (2) de la participation de tous les auteurs au manuscrit, de leur approbation de ce dernier et de l'assurance que le manuscrit en question n'est pas en cours d'examen aux fins de publications dans une autre revue, (3) de suggestions quant aux arbitres éventuels, (4) d'une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis et (5) des numéros de téléphone et de télécopieur ainsi que de l'adresse de courrier électronique de l'auteur correspondant. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les similis) jusqu'à ce qu'on les lui demande. **Afin de les protéger lors de l'expédition, les manuscrits et les illustrations doivent être solidement emballés.**

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Les auteurs sont vivement encouragés à soumettre, en plus de la copie imprimée, une copie sur disquette (3,5 ou 5,25 pouces) du manuscrit (version *acceptée* uniquement) préparée en logiciel de traitement de texte (accompagné d'une filière en format ASCII) sur ordinateur Macintosh ou sur ordinateur personnel compatible à l'IBM. Le logiciel de traitement de texte et le numéro de version doivent être identifiés.

La publication d'un article fait foi du transfert volontaire, de la part de l'auteur, de tous les droits d'auteur au CNRC. Toute permission de reproduction ou de republication d'une partie ou de la totalité d'un article doit être obtenue auprès du Service de publication du CNRC.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103, U.S.A.).C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5e éd. 1991. Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee et al. (1990. Trans. Am. Fish. Soc. 119 : 2–15). Toutes les mesures doivent être conformes au système métrique et, dans

la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, no. 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1–79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale, ON M9W 1R3, Canada).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. **Il doit éviter de modifier le contenu du texte**.

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*.

Dactylographiez le manuscrit sur papier blanc (mesurant 8,5 sur 11 pouces) sur un côté de la page. Laissez toujours une marge d'au moins 1 pouce.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à **double interligne**. Si on ne dispose pas d'une police italique, souligner les passagees à imprimer en italique.

En aucun endroit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manusrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il représentatif du contenu du rapport? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème? La section *matériel et méthodes* contient-elle le minimum de détails qui permettront aux lecteurs de saisir les grandes lignes de l'étude et de juger de la valeur des données? Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

(1) Limitez le résumé à un paragraphe d'environ 175 mots.

(2) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

(3) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

(4) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

(5) Dans les figures, les tableaux et le texte, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

(6) Évitez les formes ambiguës, telles que g C/m^2/d; utilisez plutôt g C·m^{-2}·d^{-1}.

(7) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

(8) Dans le texte, généralisez à partir des tableaux et des figures; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

(9) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

(10) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

(11) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

(12) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex. disponible auprès du Département d'économique, Université Simon Fraser, Burnaby, BC V5A 1S6, Canada.

(13) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1990) et ne soulignez pas *et al.*

(14) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

(15) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies ou des imprimés laser des figures, ou encore les originaux, s'ils ne dépassent pas 8,5 sur 11 pouces. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent

assurer, pour le lecteur, une marche logique; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : (1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et (2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à . . . » ou « Recherche sur . . . ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que « . . . on y analyse » ou « . . . a été trouvé »; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail et qu'il soit complet en lui-même (c.-à-d. seule les références *essentielles* sont indiquées, sous forme retraçable, p. ex. R.B. Deriso. 1980. J. Can. Sci. Halieut. Aquat. 37 : 268–282).

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renforme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer . . . nous . . . ». Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude; que ces réponses soient aussi complètes que possible, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les

adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. **On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa**. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½ ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des minuscules suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit « Tableau — près d'ici ».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies, des imprimés laser ou les dessins originaux (pourvu qu'ils ne dépassent pas 8,5 sur 11 pouces) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1 ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elles devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig. — près d'ici ».

Les illustrations produites par imprimantes laser sont acceptables; les illustrations produites par imprimantes par points ne sont pas acceptables. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

Nous accordons une préférence au programme de graphiques CorelDraw!, grâce auquel nous pouvons traiter divers formats compatibles à l'IBM (p. ex. .EPS, .TIF). Le nom du programme, de la version, les numéros de figure, les noms de fichier ainsi que leur suffixe doivent être clairement indiqués sur toute disquette. Utilisez les suffixes normalisés pour les programmes utilsés. Le cas échéant, il se pourrait que nous ne puissions pas afficher ou ouvrir vos fichiers.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essetielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont débités à l'auteur; le prix actuel est de 1000 $ pour chaque page ou partie de page. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleurs, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs. Pour obtenir de plus amples renseignements, communiquez avec le Bureau de la rédaction ou le Bureau des publications. On peut obtenir auprès du Bureau de la rédaction la brochure *Préparation des photographies et des illustrations en vue de la reprographie*, préparée spécialement par le Bureau des publications.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits ou souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	(an)
année	(a)
becquerel	(Bq)
calorie	(cal)
centimètre	(cm)
centimètre carré	(cm^2)
centimètre cube	(cm^3)
centimetrès par gramme par seconde	($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb	(C)
décimètre	(dm)
degré Celsius	(°C)
degrés de liberté	(dl)
écart type	(ET)
électronvolt	(eV)
erreur type	(ErT)
farad	(F)
gramme	(g)
hectare	(ha)
hertz	(Hz)
heure	(h)
joule	(J)
jour	(d)
kilomètre	(km)
litre	(L)
lumen	(lm)
lux	(lx)
mètre	(m)
mètre carré	(m^2)
mètre cube	(m^3)
micromètre	(µm)
milligramme	(mg)
millilitre	(mL)
millimètre	(mm)
millimètre carré	(mm^2)
minute	(min)

mois	(mo)
masse molaire	(M)
mole	(mol)
moles par litre	(mol/L, M)
pascal	(Pa)
seconde	(s)
semaine	(sem)
tonne (métrique)	(t)
volt	(V)
volume	(vol)
watt	(W)

Dates

On écrira les dates dans l'ordre jour-mois-année, p. ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année
Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : « Brown (1983) compare . . . » ou « . . . on a comparé (Brown 1983) ». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « et al. » : « Brown et al. (1983) comparent . . . » ou « . . . on a comparé (Brown et al. 1983) ».

Communications personnelles
Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « communication personnelle ».

Données inédites
Si un livre ou un article inédit a été accepté pour publication, insérez-le dans la liste des références, avec la mention « Sous presse ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si un article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

Les références doivent être classées par ordre alphabétique des noms premiers auteurs. Si la liste comprend plusieurs publications d'un même premier auteur, les règles suivantes seront appliquées : (1) S'il n'y a qu'**un auteur**, les ouvrages se succèdent par ordre chronologique de parution en commençant par le plus ancien. Si l'auteur a publié, au cours d'une année, plusieurs livres ou articles, on distinguera les références les unes des autres en faisant suivre l'année de publication d'une lettre (*a*, *b*, *c*, etc.). (2) S'il y a **deux auteurs**, les ouvrages sont placés à la suite de ceux n'ayant qu'un auteur, et sont alors classés par ordre alphabétique du nom du deuxième auteur. (3) S'il y a **trois auteurs ou plus**, les ouvrages sont placés à la suite de ceux ayant deux auteurs et sont classés par ordre chronologique. La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après *CASSI* ou *les Serial Sources for the BIOSIS Data Base*).

Article du journal
Archambault, D., et Bourget, E. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctiques. Can. J. Fish. Aquat. Sci. 40 : 1278–1292.

Numéro complet du journal
Gordon, D.C., Jr., et Hourston, A.S. (*Éditeurs*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Livre dans une série
Hilton, J.W., et Slinger, S.J. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. Spéc. Can. Sci. Halieut. Aquat. No. 55.

Livre ne faisant pas partie d'une série
Forrester, W.D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa, Ont.

Partie d'un livre
Aubert, M., Aubert, J., Daniel, S., et Desirotte, N. 1982. État actuel de la pollution bactérienne au large des côtes françaises. *Dans* La pollution des mers et les ressources biologiques. *Éditeur* : M. Ruivo. Fishing News (Books) Ltd., Londres. pp. 36–40.

Corporation-auteur
American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14^{e} éd. Washington, D.C.

Thèse
Delisle, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Université d'Ottawa, Ottawa, Ont.

Rapports
Dutil, D.J., Fortin, M., et Vigneault, Y. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. Manuscr. Can. Halieut. Aquat. No. 1653.

Traduction
Koike, A., et Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64 : 1–11. (Traduit du Japonais par Can. Transl. Fish. Aquat. Sci. 4950, 1983.)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles « h », « min » et « s » ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12 : 30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, « cold water » (n.), « cold-water » (adj.), « hard water » (n.), « hardwater » (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

NRC Research Press Publication Policy

1. Mandate

The National Research Council Act confers on the National Research Council of Canada (NRC) the right to publish scientific and technical information.

The objective of NRC Research Press is to publish high-quality scientific and engineering journals and monographs in a timely and cost-effective manner. The research journals and monographs published by NRC provide scientists and engineers in Canada and elsewhere with a means to communicate with their peers in the international research community. Both the Editors and NRC Research Press are committed to producing high-quality international publications, thereby adding a Canadian contribution to the world's research literature.

2. Rationale

Since NRC began the publication of research journals in 1929, numerous editorial practices and procedures have been developed on the basis of time-honoured traditions of the various disciplines. It is important that NRC follow the best current practices, and where possible, improve on them. This document reviews and formalizes the editorial and production policies and defines a set of ethical publication guidelines. Adherence to these policies will protect the integrity of the process of publication and the reputation of NRC as a publisher.

3. Scope

The policies outlined in this document are intended to cover all aspects of the NRC Research Press program.

This document begins with a description of the responsibilities of authors, editors, the Editor-in-Chief, reviewers, and the publisher. The section on publishing policy treats areas such as copyright, language of publication, the use of SI units (Système international d'unités), and advertising policy.

4. Responsibilities

Authors

1. Authors have an obligation to present an accurate account of the research performed and are responsible for complete reporting of the observations made and data collected.

2. Authors must relate their work to that of others and provide complete and accurate citations so that the readers can objectively evaluate the paper.

3. The authors should describe the safeguards used to meet both formal and informal standards of ethical conduct of research (approval of a research protocol by an institutional committee, procurement of informed consent, proper treatment of animals, and maintenance of confidentiality of personal data on patients, etc.).

4. It is the responsibility of authors to ensure that manuscripts accepted for publication are free of any kind of prejudice, especially gender and racial stereotyping.

5. Co-authors of a paper should be limited to those who have made significant scientific contributions to the work reported. All authors of a paper should be able to take public responsibility for the content. All additional contributions should be indicated in a footnote or in the Acknowledgements section.

6. Authors should avoid fragmentation of research reports or submission of trivial reports, as this consumes excessive journal space and burdens the review process unnecessarily.

7. The corresponding author must give assurance that all co-authors have read and approved the manuscript as submitted. When dealing with manuscripts with more than one author, the Editor assumes that the corresponding author is responsible on behalf of the group.

8. The authors are responsible for obtaining any formal or informal approval or clearance of the paper from their institution or company before it is forwarded to NRC Research Press.

9. Authors must identify the sources of all information quoted and material obtained privately.

10. When a manuscript contains material (tables, figures, charts, etc.) that is protected by copyright, it is the obligation of the author to secure written permission from the holder of the copyright. Letters of permission must be sent to the Editorial Office before final acceptance of the manuscript.

11. The authors are responsible for disclosing any information that may affect the acceptance or rejection of the paper. This includes indicating if the work has been previously presented in any format (conference proceedings, abstract publication, etc.) and submitting a list of related manuscripts that the author has in press or under consideration by another journal. The paper will be considered for publication only with the understanding that it has not already been submitted to, accepted by, or published in another journal.

Editors

1. The Editor(s) is(are) responsible for the scientific calibre of the contents of their journal.

2. The Editor appoints an editorial board for the journal, subject to review by the Editor-in-Chief. The form and functioning of this board is at the discretion of the Editor, but it is usually composed of Associate Editors, who assist the Editor in assessing articles submitted for publication and act as an advisory group for the Editor as required.

3. Editors are appointed for a fixed renewable term by the Editor-in-Chief, normally 5 years.

4. The Editor may appoint one or more Assistant Editors, who may assume the Editor's responsibilities during an absence. If this absence is expected to be prolonged, an Editor pro tem may be appointed by the Editor-in-Chief.

5. It is the responsibility of the Editor to ensure that manuscripts accepted for publication meet the guidelines established for authors.

6. The Editor is responsible for ensuring that proper review procedures are followed and for making the final decision on acceptance or rejection of a manuscript. The Editor may delegate some duties to Assistant or Associate Editors, or to a temporary Editor appointed to oversee a special issue or section, but the final responsibility remains with the Editor.

7. The peer review process is carried out under the direction of the Editor. When a manuscript is deemed appropriate for consideration for publication, arrangements are made for it to

be reviewed by at least two referees.

8. Referees are advisory to the Editor and do not make decisions on acceptance or rejection of a manuscript. The name of a referee will only be conveyed to an author with the explicit consent of the referee.

9. The Editor must ensure that submitted papers are dealt with in a timely manner. The Editor will endeavour to notify all authors of the receipt of the manuscript. The Editorial Office will ensure that the corresponding author is notified of any changes in status of the manuscript and all decisions that follow.

10. The Editor is responsible for ensuring that the contents of an author's paper are not divulged to anyone other than persons necessarily involved in reading the paper in the Editorial Office or reviewing it for the Editor or persons assisting in these functions.

11. The Editor should give unbiased consideration to all manuscripts and should declare any potential conflict of interest, in which case the Editor or Editor-in-Chief should delegate the consideration of the manuscript to another Editor or to a member of the Editorial Board.

12. All files regarding an individual manuscript are the property of the Editorial Office and are to be retained by the Editor or disposed of in consultation with the Editor-in-Chief.

13. When notified of errors in facts or conclusions in a paper subsequent to its publication, the Editor, after notifying the corresponding author, is responsible for initiating publication of an erratum or a report pointing out the changes.

14. If the Editor receives convincing evidence of plagiarism, duplicate publication, or falsification of data, the Editor must initiate publication of a retraction. The Editor must notify the Editor-in-Chief as soon as possible of any serious breaches of ethical behaviour.

Referees

1. Referees are advisors to the Editor and should serve only in their areas of expertise. A referee who feels inadequately qualified to evaluate a manuscript should return it promptly to the Editor.

2. A referee who cannot give an unbiased opinion about a manuscript should declare this bias or conflict of interest to the Editor and return the manuscript promptly.

3. Referees must treat the paper as a confidential communication. If a referee wishes to seek expert advice from an associate, he/she should consult the appropriate Editor or Associate Editor before proceeding. The associate must also honour the confidentiality of the document.

4. A referee who is unable to complete the review of a manuscript in an appropriate time frame should return it to the Editor promptly.

5. All statements made by the referee must be adequately supported so that the Editor may make a well-informed decision regarding the manuscript.

6. The referee should call to the attention of the Editor any failure by an author to cite relevant work by other scientists.

7. Unpublished information, arguments, or interpretations disclosed in a submitted manuscript should not be used for the referee's own research except with the consent of the author.

Editor-in-Chief

1. The Editor-in-Chief has overall responsibility for the scientific and editorial integrity of the journals and monographs published by the NRC Research Press program.

2. Questions regarding the scientific quality and competitiveness of the publications, as well as ethical questions (e.g., reporting of fraudulent data, duplicate publication, submission of inappropriate material), may be referred to the Editor-in-Chief.

3. The Editor-in-Chief is responsible for selecting Editors for the journals and an Editorial Board for the monograph program. The Editor-in-Chief consults broadly and may seek advice from other Editors, from the relevant scientific and engineering societies, and from relevant Editorial Boards regarding selection of a new Editor.

4. In cases where Editors are not fulfilling their obligations as outlined in this policy, the Editor-in-Chief has the authority to terminate appointments.

5. The Editor-in-Chief has the right of access to all files maintained by the Editors of NRC Research Press' publications. The Editor-in-Chief may exercise this right when necessary to ensure that the policies and procedures of the Editorial Offices conform to the requirements outlined in this Publication Policy.

Publisher

1. The Publisher, represented by the Director of NRC's Research Press program, is responsible for setting policies, procedures, and guidelines and for production of the journals through the Publishing Office.

2. The Publishing Office must ensure that confidentiality of the manuscripts is maintained at all stages of the pre-publishing process. Information must not be given to persons unauthorized by the author (reporters for the news media or employees of investment firms, for example) prior to the appearance of the paper in print.

3. After a manuscript has been accepted for publication, the Editor or copy editor may make redactorial changes as required but must not make substantive changes in the content of a paper without consultation with the author and the Editor.

4. The staff of the Publishing Office must alert the Editor or Editor-in-Chief concerning any substantive changes requested by an author after acceptance of the manuscript, including changes in authorship or deletion or insertion of substantial amounts of material.

5. The Publisher accepts no responsibility for statements or opinions expressed by a contributor, and a statement to this effect will appear in all publications.

5. Publishing Policy

Copyright

Once an author's paper is accepted for publication, the author transfers copyright to the National Research Council of Canada. As the holder of copyright on all original material published in the NRC journals, NRC exercises the right to grant permission to copy or republish articles or portions thereof, provided that the source of such material is fully acknowledged.

NRC reserves the right to charge for all forms of copying or publishing, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale.

Publishing in Both Official Languages

The NRC Research Press program of the National Research Council of Canada follows the guidelines on publishing in both official languages as outlined by the Treasury Board of Canada Secretariat (see Circular No. 1982-58, section 10). Briefly, an article is published in English or French according to the author's choice, preceded by an informative summary or abstract in both English and French.

Standard Units

In the NRC journals, metric units are used or the metric equivalents given, and the use of SI units is encouraged, as outlined in the Canadian Metric Practice Guide (CAN/CSA -Z234.1-89).

Advertising in NRC Journals

Advertising is accepted subject to approval by the Business Manager. All advertising must be consistent with the editorial policy and no advertisement that is detrimental to NRC or NRC Research Press will be accepted. The following types of advertising are accepted:

(i) Advertisements submitted by other scholarly journals may be accepted free of charge where benefit is expected for NRC publications in exchange.

(ii) Advertisements announcing upcoming conferences and symposia may be accepted free of charge, subject to approval by the Editor of the publication concerned. Such advertisements will only be accepted when the event is of interest to a significant proportion of the Canadian scientific community served by that publication. The event need not take place in Canada.

(iii) Paid advertisements may be accepted providing they are consistent with the editorial policy of the publication. No advertising that implies endorsement of a product or service by the publication, the Editorial Board, or NRC will be accepted. Loose inserts are considered to be paid advertisements.

List Rental

NRC Research Press makes available, for rental on a one-time-use basis only, the lists of subscribers to the journals. No subscriber is included on such a list when that subscriber or society has requested non-inclusion.

Government policy

All copy and visual material will conform to Treasury Board guidelines for the elimination of sexual and racial stereotyping. These are enunciated in the Treasury Board Administrative Policy Manual, Chapter 484, and Treasury Board Circular No. 1982-50, *Elimination of Sexual Stereotyping*, and No. 1984-4, *Guidelines for the Representative Depiction of Visible and Ethnic Minorities and Aboriginal Peoples in Government Communications*.

Presses Scientifiques du CNRC
Politique de Publication[1]

1. Mandat

La Loi sur le Conseil national de recherches confère au Conseil national de recherches du Canada (CNRC) le droit de publier des documents scientifiques et techniques.

Le programme des Presses scientifiques du CNRC a pour objectif de publier, au moment opportun et de façon rentable, des revues et des monographies scientifiques et techniques de haute qualité. Les monographies et les revues scientifiques publiées par le CNRC permettent aux scientifiques et aux ingénieurs canadiens et du monde entier d'établir des liens avec leurs collègues de la communauté scientifique internationale. Les Presses scientifiques du CNRC et leurs directeurs scientifiques ont pour mandat de produire des publications internationales de haute qualité, ajoutant ainsi une contribution du Canada à l'enrichissement de la documentation scientifique mondiale.

2. Raison d'être

Depuis que le CNRC a entrepris la publication de revues scientifiques en 1929, de nombreuses méthodes et procédures éditoriales ont été élaborées en suivant les usages propres à chacune des diverses disciplines. Il est important que le CNRC mette en oeuvre les méthodes déjà éprouvées dans ce domaine et, lorsque cela est possible, améliore ces méthodes. Le présent document récapitule et officialise les principes directeurs qui régissent l'édition et la production des documents scientifiques et définit une série de règles de conduite en matière de publication. La conformité à ces principes directeurs aura pour effet de protéger l'intégrité du processus de publication et la réputation du CNRC à titre d'éditeur.

3. Portée

Les principes directeurs contenus dans le présent document s'appliquent à tous les aspects du programme des Presses scientifiques du CNRC.

On y trouve la description des responsabilités des auteurs, des directeurs scientifiques, du directeur général des Revues scientifiques, des examinateurs et de l'éditeur. Les questions du droit d'auteur, de la langue de publication, de l'emploi du Système international d'unités (SI) et des règles en matière de publicité font l'objet de la section Politique de publication.

4. Responsabilités

Auteurs

1. L'auteur d'un article a l'obligation de fournir un compte rendu exact des travaux de recherche exécutés et de rapporter de façon complète les observations effectuées et les données recueillies.

2. L'auteur doit mettre ses travaux en rapport avec ceux d'autres chercheurs et fournir des références complètes et exactes de façon à ce que le lecteur puisse évaluer l'article d'une manière objective.

3. L'auteur doit décrire les mesures qui ont été prises dans le but de satisfaire aux normes formelles et informelles de l'éthique régissant la recherche (autorisation d'un protocole de recherche par un comité de l'établissement, obtention d'un consentement éclairé, traitement approprié des animaux et confidentialité des données personnelles sur les malades, etc.).

4. Il appartient à l'auteur de s'assurer que le langage utilisé dans son article ne soit pas entaché de préjugés, et en particulier que l'article ne contienne pas de stéréotypes sexuels ou raciaux.

5. Seule une personne ayant contribué de façon importante du point de vue scientifique aux travaux rapportés peut être désignée coauteur d'un article. Tous les auteurs d'un article doivent être en mesure d'assumer la responsabilité publique du contenu de cet article. Toutes les autres contributions peuvent être signalées dans une note infrapaginale ou dans la section Remerciements.

6. L'auteur devrait s'abstenir de fragmenter un rapport de recherche et de soumettre des rapports ne présentant aucun intérêt particulier afin de ne pas réduire indûment l'espace de publication disponible et alourdir inutilement le processus d'évaluation.

7. L'auteur correspondant doit donner l'assurance que tous les coauteurs ont lu et approuvé le manuscrit tel qu'il a été soumis. Lorsqu'un manuscrit comporte les noms de plusieurs auteurs, le directeur scientifique tient pour acquis que l'auteur qui soumet l'article est responsable pour le groupe.

8. Il appartient à l'auteur d'obtenir auprès de son établissement ou de son entreprise tout consentement ou autorisation nécessaire avant de soumettre un article aux Presses scientifiques du CNRC.

9. L'auteur a l'obligation d'indiquer la source de toute information citée et de tous documents dont il a obtenu copie à titre personnel.

10. Lorsqu'un manuscrit renferme des données (tableaux, figures, schémas, etc.) protégées par le droit d'auteur, il appartient à l'auteur de l'article d'obtenir la permission écrite du détenteur du droit d'auteur. Les lettres d'autorisation doivent parvenir au Bureau de la rédaction avant l'étape de l'approbation finale du manuscrit.

11. L'auteur est tenu de divulguer tout renseignement pouvant avoir une incidence sur la décision d'accepter ou de refuser l'article. Il doit donc indiquer si les travaux rapportés ont déjà fait l'objet d'une quelconque présentation (comptes rendus de conférence, résumés, etc.) et fournir la liste des articles associés à ces travaux et sur le point d'être publiés ou qui ont été soumis à une autre revue scientifique. L'article ne sera évalué aux fins de publication qu'à la condition de n'avoir pas déjà été proposé à une autre revue, accepté pour publication ou publié par une autre revue scientifique.

Directeurs scientifiques

1. Le(s) directeur(s) scientifique(s) est (sont) responsable(s) du maintien du caractère scientifique du contenu de la revue dont il(s) a (ont) la charge.

1. Dans le présent document, à moins d'indication contraire dictée par le contexte et pour éviter d'alourdir le texte, l'emploi du masculin comprend le féminin.

2. Les membres du comité de rédaction d'une revue sont nommés par le directeur scientifique avec l'approbation du directeur général. La structure et le fonctionnement du comité sont généralement laissés à la discrétion du directeur scientifique; toutefois, un comité de rédaction est habituellement composé de directeurs scientifiques associés dont la tâche consiste à prêter main-forte au directeur scientifique concernant l'évaluation des articles soumis pour publication et à le conseiller si besoin est.

3. Les directeurs scientifiques sont nommés par le directeur général pour des mandats renouvelables fixes, d'une durée habituelle de 5 ans.

4. Le directeur scientifique a le loisir de nommer un ou plusieurs directeurs scientifiques adjoints qui peuvent assumer ses fonctions en son absence. S'il est prévu que cette absence se prolongera, le directeur général pourra nommer un directeur provisoire.

5. Il appartient au directeur scientifique de s'assurer que tous les manuscrits acceptés respectent les directives à l'intention des auteurs.

6. Il revient au directeur scientifique de veiller à ce que les manuscrits soient évalués selon les règles et de prendre la décision finale quant à l'acceptation ou au rejet d'un manuscrit. Le directeur scientifique peut déléguer certaines de ses tâches aux directeurs scientifiques adjoints ou associés ou à un directeur scientifique provisoire nommé pour coordonner la publication d'un numéro spécial ou d'une rubrique particulière; en dernière analyse, le directeur scientifique demeure toutefois le seul responsable de la Revue.

7. Le directeur scientifique dirige le processus d'évaluation des manuscrits par les pairs. Un manuscrit qui répond aux exigences du processus d'évaluation doit être examiné par au moins deux examinateurs.

8. Les examinateurs n'ont qu'un pouvoir consultatif auprès du directeur scientifique et ne prennent aucune décision quant à l'acceptation ou au refus d'un manuscrit. Le nom d'un examinateur ne peut être communiqué à l'auteur d'un article qu'avec le consentement explicite de cet examinateur.

9. Le directeur scientifique doit veiller à ce que les articles soumis soient traités dans des délais opportuns. Il s'efforcera de faire parvenir un accusé de réception du manuscrit à tous les auteurs. Le Bureau de la rédaction veillera à informer l'auteur correspondant de tout changement de statut du manuscrit et de toutes décisions subséquentes.

10. Le directeur scientifique doit s'assurer que le contenu d'un article n'est pas communiqué à des personnes autres que celles nécessairement mises à contribution à l'étape de la lecture du manuscrit au Bureau de la rédaction, et à celle de l'évaluation par le directeur scientifique ou les personnes qui l'assistent.

11. Le directeur scientifique doit examiner les manuscrits en toute impartialité. Il doit déclarer tout conflit d'intérêts possible. Dans un tel cas, le directeur scientifique ou le directeur général remettra le manuscrit à un autre directeur scientifique ou à un membre du Comité de rédaction.

12. Tous les dossiers associés à un manuscrit sont la propriété du Bureau de la rédaction et sont conservés par le directeur scientifique. Ce dernier peut aussi s'en départir, sous réserve de l'approbation du directeur général.

13. Il incombe au directeur scientifique d'informer l'auteur des erreurs dans les faits ou les conclusions qui pourraient lui être rapportées après la publication d'un article et de prendre les dispositions nécessaires afin de publier un erratum ou un rapport indiquant les changements.

14. S'il obtient des preuves convaincantes qu'il y a eu plagiat, publication en duplicata ou falsification de données, le directeur scientifique doit prendre les dispositions nécessaires pour publier des rétractations. Il doit informé le directeur général de tout manquement sérieux à l'éthique.

Examinateurs

1. Les examinateurs n'ont qu'un rôle consultatif auprès du directeur scientifique et ne devront opérer que dans leur domaine de compétence. Un examinateur qui s'estime insuffisamment qualifié pour évaluer un manuscrit devra le retourner promptement au directeur scientifique.

2. Un examinateur qui n'est pas en mesure de donner une opinion impartiale sur un manuscrit devra en aviser le directeur scientifique ou l'aviser de l'existence d'un conflit d'intérêts et retourner le manuscrit sans délai.

3. Les examinateurs doivent traiter les articles en toute confidentialité. Si un examinateur désire obtenir l'opinion experte d'un collègue, il devra consulter le directeur scientifique ou le directeur scientifique associé avant de poursuivre son travail. Ledit collègue devra également respecter le caractère confidentiel du document.

4. Un examinateur qui se trouve dans l'impossibilité d'achever l'évaluation d'un manuscrit dans les délais prescrits devra le retourner promptement au directeur scientifique.

5. Les avis donnés par l'examinateur devront s'appuyer sur une argumentation solide qui permette au directeur scientifique de prendre la décision qui s'impose en ce qui concerne le manuscrit.

6. L'examinateur devra porter à l'attention du directeur scientifique toute omission de la part d'un auteur de citer des travaux pertinents d'autres scientifiques.

7. L'information et les argumentations non encore publiées, ou les interprétations divulguées dans un manuscrit déjà soumis, ne devront pas être utilisées pour les propres travaux de recherche de l'examinateur sans l'autorisation de l'auteur.

Directeur général des Revues scientifiques

1. Le directeur général a la responsabilité globale de l'intégrité scientifique et éditoriale des revues et monographies publiées dans le cadre du programme des Presses scientifiques du CNRC.

2. Les questions relatives à la qualité et à la compétitivité scientifiques des publications ainsi que les questions d'éthique (données frauduleuses, publication en duplicata, remise de documents inappropriés) peuvent être soumises au directeur général.

3. Le directeur général est chargé de la sélection des directeurs scientifiques des revues et d'un comité de rédaction pour le programme de monographies. Le directeur général recueille le maximum d'avis et peut solliciter ceux d'autres directeurs scientifiques, de sociétés scientifiques et d'ingénierie, et de comités de rédaction compétents pour le recrutement d'un nouveau directeur scientifique.

4. Dans les cas où les directeurs scientifiques ne remplissent pas leurs obligations en ce qui concerne les critères énoncés dans la présente politique, le directeur général a le pouvoir d'exiger leur démission.

5. Le directeur général a droit d'accès à tous les dossiers administrés par les directeurs scientifiques des publications des Presses scientifiques du CNRC. Le directeur général peut exercer ce droit lorsque nécessaire pour s'assurer que les politiques et procédures des bureaux de la rédaction sont conformes aux dispositions énoncées dans la Politique de publication.

Éditeur

1. L'éditeur, représenté par le directeur du programme des

Revues scientifiques du CNRC, est chargé de l'établissement de politiques, de procédures et des directives ainsi que de la production des revues scientifiques par l'intermédiaire du Bureau des publications.

2. Le Bureau des publications doit s'assurer que la confidentialité des manuscrits est maintenue à toute les étapes du processus qui précède la publication. L'information ne peut être communiquée à qui que ce soit (représentants des médias ou employés de maisons d'investissement, par exemple) sans l'autorisation de l'auteur avant la publication.

3. Après acceptation du manuscrit pour publication, le directeur scientifique ou le rédacteur technique pourra éventuellement effectuer des modifications rédactionnelles mais il ne devra pas modifier substantiellement le contenu d'un article sans consulter l'auteur ou le directeur scientifique.

4. Le personnel du Bureau des publications doit aviser le directeur scientifique ou le directeur général de tout changement substantiel demandé par un auteur après acceptation du manuscrit, ceci incluant les changements de noms d'auteurs ainsi que les suppressions ou les insertions atteignant un volume substantiel.

5. L'éditeur ne se tient en aucune façon responsable des opinions ou déclarations exprimées par un contributaire, et un avertissement à cet effet figurera dans toutes les publications.

5. Politique de publication

Droits d'auteur

Dès qu'un article est accepté pour publication dans une publication du CNRC, l'auteur de cet article transfère ses droits au Conseil national de recherches du Canada. Le CNRC étant le détenteur des droits d'auteur de tous les écrits originaux publiés par les Presses scientifiques du CNRC, ce dernier se réserve le droit d'accorder la permission de copier ou de republier des articles ou des parties d'articles à la condition que la source de tel article ou partie d'article soit bien indiquée.

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Publication dans les deux langues officielles

Le programme des Revues scientifiques du CNRC suit les lignes directrices relatives à la publication dans les deux langues officielles énoncées par le Secrétariat du Conseil du Trésor du Canada (dans sa circulaire n° 1982-58, article 10). En résumé, un article est publié en français ou en anglais, au gré de l'auteur, et il est précédé d'un résumé ou d'un extrait instructif en français et en anglais.

Système d'unités

Dans les Revues scientifiques, on utilise les unités métriques ou on donne les équivalents métriques et l'emploi des unités SI est encouragé, comme indiqué dans le *Guide canadien de familiarisation au système métrique* (CAN/CSA-Z234.1-89).

Publicité dans les Revues scientifiques

L'acceptation de publicité dans les Revues scientifiques est sujette à l'approbation du Responsable des opérations. Toute publicité doit se conformer aux dispositions de la politique éditoriale et aucune publicité contraire aux intérêts du CNRC ou des Revues scientifiques ne sera acceptée. Les types suivants de publicité pourront être acceptés :

(i) La publicité provenant d'autres revues savantes pourra être acceptée gratuitement chaque fois que les Revues scientifiques du CNRC pourront bénéficier d'une contrepartie ou d'un échange.

(ii) Les publicités annonçant des conférences et colloques à venir pourront être acceptées gratuitement sous réserve de l'approbation du directeur scientifique de cette publication. Ces publicités relatives à des conférences et colloques ne seront acceptées que lorsque l'évènement présentera un intérêt pour une fraction substantielle de la communauté scientifique canadienne desservie par la publication. Il n'est pas nécessaire que l'évènement ait lieu au Canada.

(iii) Les publicités payées peuvent être acceptées à condition qu'elles se conforment aux dispositions de la politique éditoriale de la publication. Aucune publicité impliquant l'approbation d'un produit ou d'un service par la publication, le Comité de rédaction ou le CNRC ne sera acceptée. Les encarts mobiles sont considérés comme une forme de publicité payée.

Location des listes

Les Revues scientifiques du CNRC louent la liste des abonnés aux revues, à condition que celle-ci ne soit utilisée qu'une seule fois. Aucun abonné ne figurera sur cette liste quand cet abonné, ou cette société s'il s'agit d'une société, aura demandé d'en être exclu.

Politique gouvernementale

Toutes copies et tous documents visuels se conformeront aux lignes directrices du Conseil du Trésor pour l'élimination des stéréotypes sexuels et raciaux. Ceux-ci sont énoncés dans le manuel de la politique administrative du Conseil du Trésor au chapitre 484 et dans les circulaires n° 1982-50, *Élimination des stéréotypes sexuels,* et n° 1984-4, *Lignes de conduite touchant la représentation des minorités visibles et ethniques et celle des autochtones dans les communications gouvernementales,* du Conseil du Trésor.

39TH ANNUAL MEETING
39e CONGRÈS ANNUEL

CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES

FÉDÉRATION CANADIENNE DES SOCIÉTÉS DE BIOLOGIE

LONDON CONVENTION CENTRE
LONDON, ONTARIO
JUNE 19 - 22, 1996

PLENARY LECTURER / CONFERENCE PLENIERE
Thursday, June 20, 8:00 p.m. to 9:00 p.m.
Dr. Frederick Cornhill, The Cleveland Clinic Foundation
Biomedical Engineering Approaches in the Study of the Cardiovascular System

PMAC KEYNOTE LECTURE SERIES
Thursday, June 20, 11:30 a.m. - 12:30 p.m.
Dr. Remi Quirion, Douglas Hospital Research Centre
Alzheimer's Disease: Are There Any Links Between Beta-Amyloids, Apolipoprotein E and Cholinergic Deficits?

Friday, June 21, 11:30 a.m. - 12:30 p.m.
Dr. Ernst Niebur, California Institute of Technology
Modeling of Visual Selective Attention

Saturday, June 22, 11:30 a.m. - 12:30 p.m.
Dr. Joel Weiner, University of Alberta
Engineering of Cofactor Binding Sites in DMSO Reductase of E.Coli

THEME I - MEMBRANE BIOGENESIS AND FUNCTION
Lipid Transport and Membrane Assembly
Energy Conserving Membrane Proteins
Ion Channels, Receptors, and G Proteins

THEME II -MODELING OF BIOLOGICAL SYSTEMS
Modeling of Bimolecular Systems

THEME III -CARDIOVASCULAR SCIENCES
Myocardial Ischemia and Reperfusion: Mechanisms of Injury and Protection
New Dietary Strategies for the Prevention and Management of Cardiovascular Disease

THEME IV - NEUROBIOLOGY
Development, Growth and Regeneration in the Nervous System
Cellular and Molecular Aspects of Neurodegeneration in Alzheimer's Disease
Selective Vulnerability of Neuronal Populations to Disease: Metabolic and Environmental Factors

OTHER SYMPOSIA
New Frontiers in Medical Imaging

MINI-SYMPOSIA

Lipoprotein and Lipid Metabolism
Nuclear Organization in Cell Growth, Death, and Differentiation
Protein Structure and Function
Gene Regulation at the Fetal-Maternal Interface
Membrane Transport Processes
Signal Transduction
Protein and Amino Acid Metabolism
Biology of Tumor Progression and Metastasis

POSTER DISCUSSION WORKSHOPS

Skeletal Muscle
Digital Imaging

Abstract Deadline:
January 12, 1996

For further information: CAN FEDERATION OF BIOLOGICAL SOCIETIES
Pour plus d'information: 104 - 1750 Courtwood Cres., Ottawa, Ontario K2C 2B5
Tel: (613) 225-8889 Fax: (613) 225-9621 E-Mail cfbs@hpb.hwc.ca

DECISION MAKING AND RISK ASSESSMENT IN BIOLOGY

June 24-28, 1996
Dunedin, New Zealand,

Following the successful first conference on Statistics in Ecology and Environmental Monitoring (SEEM 1) held in at the University of Otago, Dunedin in December 1993, it is proposed to have a second conference with the general theme of Decision Making and Risk Assessment in Biology .

As with the first conference, SEEM 2 is intended to bring together biomathematicians, ecologists, environmental scientists, resource managers and statisticians with a common interest in the application of statistical methods to ecological and environmental problems, in the expectation that this interaction will be beneficial to all participants. In particular, SEEM 2 will provide a summary of the latest advances in the use of statistical methods in risk assessment at a level suitable for both scientists and managers.

The programme for SEEM 2 is still being developed. At the time of writing the following individuals have agreed to take part as Invited Speakers or Session Organizers for specific themes:

- *General Principles of Risk Assessment:* Hugh Possingham, Department of Environmental Science, University of Adelaide, Australia.
- *Border Security:* Stuart MacDiarmid, Ministry of Agriculture and Fisheries, New Zealand.
- *Endangered Populations:* Elisabeth Slooten, Department of Zoology, University of Otago.
- *Fisheries:* Chris Francis, National Institute for Water and Atmospheric Research, New Zealand; Grant Thompson, National Marine Fisheries Service, Seattle, USA.
- *Hazardous Waste:* Philip Dixon, Savannah River Ecology Laboratory, University of Georgia, USA; Richard Gilbert, Battelle-Northwest, Washington, USA.
- *Pest Management:* Murray Efford, Landcare Research, New Zealand.

Contributed papers on any quantitative aspect of biological risk assessment may be presented at the conference. Abstracts should be sent to the Conference Administrator at the address shown below by 1 March 1996. Selected papers will be published in the conference Proceedings. Authors wishing their papers to be published should submit two copies of the full paper to the Conference Administrator by 31 May 1996. The format of papers should follow the style of the 1995 volume of the journal Biometrics. All papers will be refereed using standard procedures.

Further Information

For more information about SEEM 2 contact the Conference Administrator, Centre for Applications of Statistics and Mathematics, University of Otago, P.O. Box 56, Dunedin, New Zealand (Telephone 03-479-7774, Fax 03-479-8427, E-mail casm@maths.otago.ac.nz).

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Bryan A. Henderson, Jonathan L. Wong, and Stephen J. Nepszy	**127–133**	Reproduction of walleye in Lake Erie: allocation of energy
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J. Kevin Craig, Chris J. Foote, and Chris C. Wood	**141–147**	Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*)
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Kristina M. Miller, Ruth E. Withler, and Terry D. Beacham	**181–195**	Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation
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National Research Council Canada
Ottawa, Canada K1A 0R6

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Ottawa, Canada K1A 0R6

Canadian Journal of Fisheries and Aquatic Sciences

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